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OCT 31 1935

VOLUME 25 NUMBER 10

OCTOBER, 1935

Phytopathology

An International Journal

Official Organ of
The American Phytopathological Society

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Price { $6.00 per year, United States and dependencies,
$6.25 per year, Canada
$6.50 per year, elsewhere

PUBLISHED MONTHLY BY THE SOCIETY

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COR. LIME AND GREEN STREETS, LANCASTER, PA.

Entered at the post-office in Lancaster, Pa., as second-class matter.

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PHYTOPATHOLOGY

VOLUME 25 NUMBER 10

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CHEMICAL STUDIES ON THE VIRUS OF TOBACCO MOSAIC

IV. SOME EFFECTS OF DIFFERENT CHEMICAL AGENTS ON INFECTIVITY[1]

W. M. STANLEY

(Accepted for publication August 5, 1935)

INTRODUCTION

The effect of various chemical agents on the infectivity of tobacco-mosaic virus has been studied by many workers. The earlier investigators, including Koning (18), Heintzel (10), Allard (1, 2), and Chapman (4), appear to have been interested in obtaining an insight into the nature of viruses by studying the effect of various chemicals on infectivity. The most extensive experiments were those of Allard, who found that, with the exception of acids, bases, potassium permanganate, zinc chloride, copper sulphate and antiformin in concentrations of 1 per cent or greater, all of the many chemicals which he tested left the virus preparations infectious. He also found that the virus was adsorbed on aluminum hydroxide. Somewhat later Duggar and Armstrong (6) found that pokeweed juice had an inhibiting effect on the infectivity of tobacco-mosaic virus. McKinney (21) reported similar results with juice from cucumber plants. Vinson and Petre (33) found that suspended matter tended to cause an increase in the infectivity of tobacco-mosaic virus. Johnson (15) reported that bubbling air or oxygen through preparations for several weeks attenuated tobacco-mosaic virus. Johnson (14, 16) and Johnson and Grant (17) studied the effect of ethyl alcohol, nitric acid, and formaldehyde on several viruses and suggested that reaction to chemicals might be used as an aid in classification. Johnson also pointed out the possibility of separating mixtures of viruses by the use of chemicals having a toxic or inactivating action on one of the components. Fukushi (8) found that tobacco-mosaic virus was very resistant to many agents such as aconitine, morphine, strychnine, saponin, and digitalin, and was rendered noninfectious by oil of mustard, nicotine, and atropine. Fukushi (9) also reported that tobacco-mosaic virus was readily adsorbed on kaolin, aluminia, and aluminum hydroxide gel. Henderson (11) re-

[1] Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by date of receipt of the manuscript.

ported that phenol reduced the rate of inactivation of tobacco-ring-spot virus and Bald and Samuel (3) found that sodium sulphite retarded the inactivation of tomato-spotted-wilt virus. Burt Johnson (13) reported that, of a number of salts tested, only ammonium sulphate was useful in salting tobacco-mosaic virus out of solution. Shapovalov (26) recently reported that cobalt, nickel, and zinc sulphates, at certain concentrations, appeared to be somewhat toxic to both component viruses of tomato "combination streak," so that occasionally one or the other survived, thus allowing a separation. Mulvania (22), Purdy (24), Matsumoto (20), Silberschmidt (27), and Chester (5) found that both normal and immune serum of rabbits reduced the infectivity of tobacco-mosaic virus, and Chester reported that, in the case of normal serum and certain other proteins, the reduction was due to a decrease in the susceptibility of the host.

Studies on the effect of chemicals on viruses have been directed toward 3 objectives: firstly, as an aid in gaining an insight into the nature of viruses; secondly, as an aid in the classification of viruses; and thirdly, as an aid in the separation of mixtures of viruses. In practically all cases, experiments have been made by adding chemicals to virus preparations and then determining the infectivity of the resulting mixtures at once and after given periods of time. It has been the general practice to assume that virus is inactivated whenever the mixtures fail to prove infectious. However, it has been shown recently (28) that the loss of infectivity, which occurs when trypsin or certain other proteins are added to tobacco-mosaic and other viruses, is due to inhibition of infectivity and not to inactivation of virus, inasmuch as on the removal of such proteins the virus again manifests its infectivity. As was pointed out, it seems likely that at least part of this inhibition is due to some effect on the cells of the test plant used to measure the infectivity of the mixture. It is possible that other chemicals may have a similar inhibitory action, and hence that many chemicals which, in the past, have been regarded as possessing a toxic or inactivating influence on virus, may be, in fact, only inhibitory agents. This could, of course, easily lead to erroneous conclusions regarding the nature of viruses, and to erroneous views regarding their relationships. It seemed desirable, therefore, to determine whether or not loss of infectivity on addition of different chemicals is due to inactivation of virus or to an inhibitory action. It was also desirable to gain some idea concerning chemicals having little or no effect on virus, which might be useful in connection with work on concentration and purification of virus. Furthermore, it appeared likely that a study of virus in the presence of different chemicals would yield information concerning the general nature of the virus of tobacco-mosaic, and indicate whether or not virus acts as a protein. The present paper records the results of tests with 110 different chemicals.

MATERIALS AND METHODS

All chemicals used in the experiments to be reported were of reagent quality. Samples of purified virus, prepared by a modification of the method of Vinson and Petre (33) from juice of mosaic-diseased plants of *Nicotiana tabacum* L. variety Turkish were used in all experiments, unless otherwise specified. The purified virus preparations were diluted with 0.1 M phosphate buffer at pH 6.8 to 1:10 or 1:100 in order to facilitate counting by reducing the average number of lesions produced. To 10 cc. or larger portions of diluted purified virus was added, with vigorous stirring, 1 per cent by weight, unless otherwise specified, of the chemicals to be tested. These mixtures were then immediately tested against a control for virus infectivity by the half-leaf method previously described (12, 25, 28). These tests for virus infectivity were made by comparing the average number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* L. or 12 or more half-leaves of *Phaseolus vulgaris* L. variety Early Golden Cluster on inoculation with the preparations containing various chemicals, with the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation containing no chemical. After standing 3 days at 5° C., the preparations were again tested for virus infectivity. Whenever mixtures containing chemicals produced about the same number of lesions as the control at once and after 3 days, it was assumed that the chemicals had affected neither the virus nor the plant used in measuring infectivity. When the mixtures produced about the same number of lesions as the control at once and a decreased number after 3 days, evidence was brought that the chemical did not affect the plant but that it slowly inactivated the virus. However, when mixtures produced no lesions, or produced a decreased number of lesions as compared with the control at once and after 3 days, it is impossible to determine whether the reduction was due to inactivation of virus by the chemicals or to an inhibitory effect on the plant which prevented the virus from manifesting its infectivity. In the majority of such cases, however, it was found possible to differentiate between these two possibilities by a method involving the removal of chemicals from the mixtures by dialysis. The preparations from which chemicals had been removed by dialysis were tested for virus infectivity against dialyzed controls. Any change in infectivity was assumed to have been caused by the action of the chemical on the virus. The samples to be dialyzed were placed in 4- to 24-inch lengths of ¾-inch transparent seamless Du Pont cellophane tubing containing a small glass marble and tied at either end with linen thread. The dialyses were carried out using a Kunitz and Simms (19) rocking apparatus and dialyzing against running distilled water for 4 hours, or until tests indicated that the chemical had been removed.

EXPERIMENTAL

Action of Different Chemicals on Tobacco-mosaic Virus

Table 1 records the results obtained with chemicals which were found to have little or no effect on the infectivity of the virus when tested in the presence of the reagents immediately after their addition and after standing for 3 days at 5° C. The fact that there was but slight change in the number of lesions is an indication that this group of 41 different chemicals has no appreciable effect on the virus or on the *Nicotiana glutinosa* plants used to measure its infectivity. Plants of *Phaseolus vulgaris* appeared to be affected by some of the reagents, especially those causing an acid reaction, such as citric acid, phthalic acid, and phenol. It has already been demonstrated (29) that an increase in the acidity of virus preparations beyond about pH 5 causes a marked reduction in the number of lesions produced on *P. vulgaris,* and but only a slight change in the number of lesions produced on *N. glutinosa.* For this reason the results obtained with *N. glutinosa* are regarded as the more significant. It may be seen from table 1 that, in general, the numbers of lesions produced by the chemical-containing virus preparations are about the same as the numbers produced by the control preparation.

Results obtained with a few chemicals that were found to cause an increase in virus infectivity are given in table 2. Certain of these reagents, such as dipotassium phosphate, sodium thiosulphate, phenolphthalein, and pyridine, cause a decrease in the hydrogen-ion concentration. This is probably the cause of the increased infectivity, since it has been demonstrated (29) that a decrease in the hydrogen-ion concentration from about pH 7 to pH 8 or 9 causes an increase in virus infectivity. Other of the reagents, such as benzidine, l-menthol, and sulphur, which are relatively insoluble, probably cause an increase in infectivity through their action as suspended particles.

The results obtained with a group of some 40 different reagents which were found to cause a decrease in the infectivity of virus are given in table 3. The hydrogen-ion concentration of the mixture of chemical and virus solution is given in most instances. It may be seen that, with perhaps one exception, the chemicals are either oxidizing agents, protein-precipitating agents, or reagents causing a hydrogen-ion concentration injurious to virus, test plant, or both. It is known (29) that reactions greater than about pH 10 and less than about pH 3 cause inactivation of virus, hence the hydrogen-ion concentration caused by reagents such as bromine, diisoamylamine, ferric chloride, oxalic acid, picric acid, stannic chloride, and platinic chloride would alone be sufficient to account for the inactivation. The oxidizing agents, including hydrogen peroxide, chloramine-T, potassium

TABLE 1.—*Infectivity tests on purified virus preparations immediately after the addition of various chemicals and after standing for 3 days at 5° C. Reagents in this group were found to have but slight effect on the infectivity of the virus*

Chemicals tested	Tested immediately after mixing				Tested after 3 days at 5° C.			
	Phaseolus vulgaris		Nicotiana glutinosa		Phaseolus vulgaris		Nicotiana glutinosa	
	Chemical	Chemical ÷ control	Chemical	Chemical ÷ control	Chemical	Chemical ÷ control	Chemical	Chemical ÷ control
1% aniline	33.2[a]	60.0[b]	101.2[a]	103.0[b]	13.2	99.0	72.5	103.5
1% boric acid	26.4	65.5	47.5	130.0	12.5	100.0	112.2	105.5
0.1% bromcresol purple	10.7	52.3	67.4	153.0	14.4	69.2	87.5	145.8
1% p-amino benzoic acid	13.0	29.5	24.6	102.5	11.0	64.0	51.0	242.0
1% butyl alcohol	36.8	64.5	119.0	92.5	7.0	55.0	49.0	98.0
1% caprylic alcohol	16.9	49.0	72.3	90.4	6.9	40.6	78.9	102.6
1% carbon tetrachloride	31.9	88.5	86.5	87.0	23.9	88.5	115.2	143.0
1% chlorobenzene	10.7	37.0	21.8	72.2	18.1	128.0	64.6	111.0
1% citric acid	1.6	8.7	59.0	79.4	1.1	7.7	58.8	110.0
0.1% cresol red	11.6	45.0	54.5	80.0	27.3	83.0	57.0	147.5
1% cyclohexanol	22.8	44.0	147.6	129.0	9.1	67.8	60.6	138.1
1% cymene	22.5	92.8	65.7	101.6	8.1	95.2	65.6	109.0
1% cysteine			141.4	69.0			38.6	97.8
1% diethylene glycol	30.0	94.0	73.1	169.0	8.7	41.7	68.4	140.0
1% 1–4 dioxane	16.1	71.5	80.0	122.5	11.0	87.4	82.0	90.6
0.3% formaldehyde	10.0	36.5	82.0	89.0	23.1	59.2	80.8	87.5
1% glycerol	16.8	73.4	38.0	77.3	16.0	84.1	124.0	87.9
1% hydrazine			88.6	57.8			76.5	78.6
1% hydroquinone	17.7	54.7	66.3	106.2	7.6	55.0	58.6	90.2
0.1% iodine	26.4	66.5	44.5	88.3	13.2	161.0	50.0	151.0
1% methanol	16.8	59.6	41.6	83.4	5.6	56.5	50.4	120.0
1% methyl iodide	28.4	112.5	141.8	135.8	7.5	36.6	53.2	77.2
1% methyl orange	16.8	86.2	33.4	76.5	7.2	25.2	56.2	103.9
1% magnesium nitrate	18.3	111.4	35.0	132.0	7.2	38.6	72.6	90.9
1% monopotassium phosphate	32.3	99.4	113.6	114.5	11.8	163.0	133.0	94.5
1% α-naphthol	18.2	71.0	56.2	98.0	8.3	37.8	75.8	145.8
1% ß-naphthol amine	25.6	70.8	39.8	46.7	16.4	94.1	86.6	99.0
1% para-formaldehyde	35.1	162.5	114.6	112.2	14.4	50.4	44.2	90.0
1% phenol	9.5	160.8	35.0	70.5	24.6	23.4	77.7	118.0
1% phthalic acid	14.1	86.5	87.0	138.8	1.9	13.5	96.6	130.4
1% potassium ferrocyanide	14.1	31.8	39.0	85.3	5.6	26.0	95.4	96.5
1% potassium thiocyanate	22.8	110.5	100.8	109.0	8.6	86.0	110.0	170.0
1% pyrogallic acid	13.4	26.8	30.1	109.5	4.0	57.0	62.8	99.3
1% resorcinol	9.6	44.5	45.0	97.8	17.1	82.2	109.2	119.5
1% rosaniline HCl	10.0	21.9	90.2	77.5	5.3	64.6	50.6	91.6
1% sodium benzoate	3.4	21.4	54.9	127.2	11.5	21.7	64.0	80.5
1% sodium hydrosulphite	23.8	105.1	94.3	116.5	9.5	100.0	61.2	124.8
1% sodium nitrite	15.7	85.5	67.0	71.6	9.4	83.1	42.0	77.5
1% sodium sulphite	32.9	105.0	35.5	93.0	39.0	83.0	32.4	41.4
1% toluidine	24.6	93.0	88.0	134.9	14.8	90.8	85.0	162.8
1% urea	12.8	48.5	114.0	111.8	7.0	64.8	82.5	106.8

[a] Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with a purified virus preparation containing the designated chemical.

[b] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 5 or more half leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the chemical-containing preparation, divided by the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation.

TABLE 2.—*Infectivity tests on purified virus preparations immedately after the addition of various chemicals and after standing for 3 days at 5° C. Reagents in this group were found to cause an increase in the infectivity of the virus*

Chemicals tested	Tested immediately after mixing				Tested after 3 days at 5° C.			
	Phaseolus vulgaris		Nicotiana glutinosa		Phaseolus vulgaris		Nicotiana glutinosa	
	Chemical	Chemical ÷ control	Chemical	Chemical ÷ control	Chemical	Chemical ÷ control	Chemical	Chemical ÷ control
1% acetal	21.6[a]	67.1[b]	133.4[a]	153.0[b]	14.3	126.7	66.0	114.1
1% ammonium sulphate	26.0	155.5	55.5	80.0	20.3	157.0	94.5	116.0
1% benzidine	19.6	68.9	112.4	113.1	14.1	133.0	74.0	140.6
1% calcium chloride	16.1	47.5	143.0	138.8	10.9	39.6	100.2	148.0
1% carbon disulphide	41.6	94.5	110.0	111.0	9.2	124.0	91.5	118.0
0.1% cresolphthalein	40.2	146.2	60.1	119.8	27.2	115.1	32.8	139.0
1% furfural	48.0	123.5	121.8	120.5	15.7	159.0	60.2	292.0
1% guaiacol	4.9	48.0	44.0	146.5	11.3	45.1	70.8	127.2
1% l-menthol	38.4	88.0	110.6	106.0	20.4	115.8	133.6	210.0
1% phenolphthalein	12.7	270.0	75.0	113.5	44.4	139.5	127.0	197.2
1% phthalimide	31.9	198.1	22.5	100.0	21.8	122.4	73.2	145.6
1% potassium chlorate	43.6	203.0	99.4	133.1	28.9	161.5	59.8	238.3
1% dipotassium phosphate	102.4	317.0	120.2	104.8	34.2	713.0	87.8	130.9
1% pyridine	15.3	106.0	92.5	144.0	38.9	112.0	80.5	119.5
1% sodium nitrate	24.8	89.0	93.0	141.0	8.2	118.7	92.0	120.2
1% sodium thiosulphate	30.9	148.5	96.6	144.0	27.4	274.0	117.6	205.0
1% sulphur	17.5	156.1	147.2	111.0	46.2	218.0	44.7	137.0

[a] Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with a purified virus preparation containing the designated chemical.

[b] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 5 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the chemical-containing preparation, divided by the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation.

permanganate, potassium dichromate, iodine, and bromine, and the protein precipitants, such as mercuric acetate and chloride, silver nitrate, lead acetate, and safranine, were found to have a marked effect in reducing the number of lesions.

It seemed desirable to determine whether the reduction in the number of lesions was caused by action of the oxidizing agents and protein precipitants on the virus or on the plant used to test infectivity, or both. Table 4

TABLE 3.—*Infectivity tests on purified virus preparations immediately after the addition of various chemicals and after standing for 3 days at 5° C. Reagents in this group were found to cause a marked decrease in the infectivity of the virus*

Chemicals tested	pH	Tested immediately after mixing				Tested after 3 days at 5° C.			
		Phaseolus vulgaris		Nicotiana glutinosa		Phaseolus vulgaris		Nicotiana glutinosa	
		Chemical	Chemical ÷ control	Chemical	Chemical ÷ control	Chemical	Chemical ÷ control	Chemical	Chemical ÷ control
1% aluminum chloride	3.5[a]	0.9[b]	4.2[c]	15.6[b]	23.3[c]	0.6	2.3	14.6	14.8
1% aluminum sulphate	3.5	0.2	1.1	33.0	36.4	0.6	5.1	14.8	21.0
10% ammonium "		12.1	46.1	11.2	16.0	6.1	32.5	30.0	33.7
50% " "		2.5	10.8	3.6	3.7	1.1	5.7	8.4	11.4
1% " sulphide	9.0	45.6	226.0	68.2	87.5	0.6	7.7	18.8	55.5
1% barbital sodium	9.0	47.8	468.0	125.2	122.0	0.4	4.2	34.2	49.8
1% benzaldehyde	3.4	9.1	26.4	87.5	116.0	0.8	4.2	74.4	70.2
Saturated bromine	2.4	0.0	0.0	1.4	1.6	0.1	0.6	4.8	4.2
0.1% bromphenol blue	4.5	31.7	87.0	69.5	95.8	7.6	33.4	25.0	33.2
1% brucine	7.6	16.4	113.0	57.5	114.0	0.0	0.0	18.5	26.4
1% chloramine-T	6.4	0.0	0.0	2.7	13.7	0.0	0.0	0.0	0.0
1% cobalt acetate	6.6	19.2	52.7	94.4	69.2	21.2	58.2	67.2	77.1
1% " sulphate				15.6	28.4				
1% copper sulphate	3.7	0.5	3.1	0.6	0.9	0.5	4.0	15.7	14.4
1% cysteine HCl	1.8	0.2	0.5	24.5	64.8	0.0	0.0	25.9	28.2
1% diisoamyl amine	10.2	18.3	79.0	15.1	31.8	0.2	1.3	5.4	11.2
1% ferric chloride	2.2	1.9	73.0	28.8	55.5	0.0	0.0	0.0	0.0
1% ferrous sulphate	4.4	2.5	12.5	74.4	95.0	1.6	10.4	39.0	55.5
1% formic acid	3.1	1.6	5.0	46.5	35.8	1.3	11.3	20.8	36.6
1% hydrazine sulphate	1.5	3.4	7.0	20.0	36.7	0.0	0.0	6.4	5.6
3% hydrogen peroxide	6.2	1.1	3.4	8.2	7.2	4.4	12.9	19.2	14.2
1% hydroxyl amine HCl	3.8	1.5	8.0	37.3	91.0	0.8	5.1	19.6	29.6
1% iodine + 1% potassium iodide	6.2	0.0	0.0	2.0	2.4	0.4	3.2	1.4	1.4
1% lead acetate	5.8	6.6	19.6	83.8	84.6	1.6	21.9	63.0	68.4
1% " subacetate	7.1	3.4	23.1	65.5	87.0	0.7	4.9	4.6	10.6
1% mercuric acetate	4.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1% " chloride	5.5	0.0	0.0	3.8	4.6	0.0	0.0	1.2	1.5
1% nickel sulphate				11.8	19.8				
1% oxalic acid	1.5	1.3	3.1	37.0	40.0	0.0	0.0	10.8	19.2
1% picric acid	1.7	5.7	14.1	36.8	48.0	0.0	0.0	0.4	0.7
1% platinic chloride	2.1	0.6	3.1	7.5	34.2	0.0	0.0	0.0	0.0
1% potassium cyanide	10.1	7.1	139.0	17.1	40.0	1.4	2.8	1.2	1.4
1% " dichromate		4.4	42.6	22.5	57.6	7.0	25.5	25.4	35.6
1% " ferricyanide	7.0	7.3	21.0	66.4	99.0	5.5	44.0	43.2	51.0
1% " permanganate	8.2	0.1	0.5	0.6	1.0	0.9	9.1	0.2	0.2
0.1% safranine	6.9	0.0	0.0	0.0	0.0	1.4	9.2	0.0	0.0
0.04% safranine	6.9	0.8	3.2	48.7	72.6	1.6	9.4	19.6	17.4
1% salicylic acid	3.1	0.3	0.6	6.2	26.2	0.1	0.4	30.4	46.9
1% silver nitrate	5.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1% sodium sulphide	11.6	0.4	0.9	0.6	1.1	0.0	0.0	1.6	1.1
1% " tungstate	7.9	22.3	78.6	65.7	77.8	0.5	7.8	23.8	29.0
1% stannic chloride	1.1	0.4	0.9	0.8	1.2	0.0	0.0	0.3	0.4
1% stannous chloride	1.6	0.2	0.9	21.6	27.8	0.2	1.1	11.8	17.6
1% sulfanilic acid		1.1	26.2	0.1	0.6	0.0	0.0	26.2	44.1
1% tannic acid	4.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1% tartaric acid	2.4	0.0	0.0	13.1	50.6	0.1	0.8	17.2	27.2
1% p-toluene sulfonic acid	1.6	3.3	16.3	15.6	21.4	0.1	0.7	1.6	3.1
1% zinc sulphate				4.6	9.2				

[a] Numbers represent the hydrogen-ion concentration of the chemical-containing virus preparations as determined using a glass electrode.

[b] Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with a purified virus preparation containing the designated chemical.

[c] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 5 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the chemical-containing preparation, divided by the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation.

records the results of an experiment in which chemicals were added to samples of a virus preparation and infectivity tests made after dialysis of the mixtures. A portion of each of the mixtures was dialyzed immediately after mixing and another portion dialyzed after standing for 3 days at 5° C. It may be seen that the reduction in number of lesions caused by oxidation is due to the action of the oxidizing agents on the virus, for after the removal of the chemical by dialysis the virus is inactive. Reversal of this inactivation by reducing agents has not been found possible, although inactivation by potassium permanganate may be interrupted by the addition of reducing agents such as cysteine. It appears that atomic oxygen is necessary in order to cause inactivation of the virus, for in an experiment in which oxygen gas was bubbled through a virus preparation by means of a sintered glass membrane, there was but a slight reduction in the infectivity of the virus after 8 days' treatment. Similar treatment with hydrogen gas for 8 days also caused but a slight reduction in the infectivity of the virus. Zinc dust at pH 3 was without effect, although sodium nitrite and sodium bisulphite at pH 3 caused a marked reduction in lesions. The two latter reagents had but slight effect at low hydrogen-ion concentrations, where the amounts of free nitrous and sulphurous acids were very small. It may also be seen from table 4 that, with the exception of mercuric acetate, the action of protein precipitants seems to be partly on the virus and partly on the plant used to measure infectivity. Cobalt, copper and zinc sulphates, potassium cyanide, and mercuric chloride appear to have but little direct effect on the virus, but a marked effect on the test plants. Silver nitrate required 3 days to cause a marked lowering of infectivity. The action of mercuric acetate, which was found to cause an immediate inactivation of the virus, was in marked contrast to that of mercuric chloride, which had but little effect even after 3 days. This may be due to the fact that mercuric acetate, which is more highly ionized than mercuric chloride, yields a greater number of reactive mercury ions. Because the fact that mercuric chloride is a powerful germicide, a detailed study of its action on virus was made.

In the first experiment, the effect of 0.5 per cent mercuric chloride on purified virus at hydrogen-ion concentrations from pH 3 to pH 8 was determined. To portions of purified virus at the various hydrogen-ion concentrations were added equal portions of 1 per cent mercuric chloride solutions at the same hydrogen-ion concentrations. Portions of each of the mixtures were immediately dialyzed against distilled water and then tested against a dialyzed control. Other portions were dialyzed at the end of various periods of time and similarly tested. The results of the infectivity tests are given in table 5. It may be seen that there is only a moderate inactivation of virus at pH 3, 4 and 5, even after long standing, but that at pH 6, 7, and 8 there is an immediate and practically complete inactivation

TABLE 4.—*Infectivity tests on purified virus preparations dialyzed after the addition of various chemicals. One portion of each of the preparations was dialyzed for 4 hours or more against distilled water immediately after the addition of the various chemicals and another portion was dialyzed after standing for 3 days at 5° C.*

Chemicals tested	pH	Dialyzed immediately after mixing		Dialyzed after standing for 3 days at 5° C.	
		Nicotiana glutinosa		Nicotiana glutinosa	
		Chemical	Chemical ÷ control	Chemical	Chemical ÷ control
1% aluminum chloride	3.5[a]	0.0[b]	0.0[c]	0.0	0.0
1% aluminum sulphate	3.5	0.0	0.0	0.0	0.0
1% chloramine-T	6.4	0.2	0.1	0.0	0.0
1% cobalt sulphate		40.2	73.3	51.4	125.5
1% copper sulphate	3.7	86.8	77.8	25.2	73.4
3% hydrogen peroxide	6.2	19.1	31.0		
1% iodine+1% potassium iodide	6.2	1.3	1.2		
1% mercuric acetate	4.1	0.0	0.0	0.0	0.0
1% mercuric chloride	5.5	94.0	66.7	34.3	70.6
1% nickel sulphate		48.9	82.3	15.0	29.3
1% potassium cyanide	10.1	132.6	91.2	117.9	90.5
1% potassium permanganate	8.2	0.0	0.0		
0.5% " "		0.0	0.0		
0.1% " "		17.2	45.8		
0.01% " "		38.9	65.0		
0.001% " "		45.0	83.1		
0.5% safranine	7.0	14.6	42.0		
1% salicylic acid	3.1			21.4	49.7
1% silver nitrate	5.1	29.9	78.0	14.6	22.4
1% sodium nitrite				116.7	74.5
1% sodium bisulphite				141.7	103.0
1% sulfanilic acid				26.2	76.1
1% tartaric acid	2.4			18.0	59.9
1% zinc sulphate				40.9	70.2
1% sodium nitrite[d]		20.1	16.1		
1% sodium bisulphite[d]		38.8	36.8		
1% zinc dust[d]		75.3	102.5		

[a] Numbers represent the hydrogen-ion concentration of the chemical-containing virus preparations before dialysis as determined by using a glass electrode.

[b] Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with a purified virus preparation containing the designated chemical.

[c] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 5 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the chemical-containing preparation, divided by the average number of lesions obtained on the other halves of the leaves on inoculation with the control preparation.

[d] Preparations at pH 3 for 3 hours and then tested in the presence of the chemicals at pH 6.5.

TABLE 5.—*The effect of 0.5 per cent mercuric chloride on purified virus preparations at various hydrogen-ion concentrations over periods of time up to 4 months at 5° C.*

pH	Tested →	At once	After 1 week	After 3 weeks	After 4 months
	Chemical	13.2[a]	8.4	21.8	0.3
3	Chemical ÷ control	83.5[b]	29.2	76.0	4.9
	Chemical	12.6	14.4	24.8	5.6
4	Chemical ÷ control	45.0	34.4	86.0	61.5
	Chemical	15.0	45.0	20.8	3.4
5	Chemical ÷ control	78.9	64.6	72.9	40.0
	Chemical	1.6	0.0	0.0	0.0
6	Chemical ÷ control	7.3	0.0	0.0	0.0
	Chemical	0.0	0.0	0.0	0.0
7	Chemical ÷ control	0.0	0.0	0.0	0.0
	Chemical	0.0	0.4	0.0	0.0
8	Chemical ÷ control	0.0	1.4	0.0	0.0

[a] Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with a purified virus preparation containing the designated chemical.

[b] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 5 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the chemical-containing preparation, divided by the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation.

of the virus. The results of a similar experiment in which untreated juice from mosaic-diseased Turkish tobacco plants was used instead of purified virus are given in table 6. It may be seen that virus in untreated juice is only moderately inactivated and that the hydrogen-ion concentration appears to have but little effect on the amount of inactivation. The results of the two experiments suggest that, when virus is present with a large

excess of extraneous protein, as is the case with untreated juice, most of the mercuric chloride is used in combining with this extraneous protein, thus leaving but a small amount to act on the virus. Therefore, the virus is affected only moderately, regardless of the hydrogen-ion concentration. However, in the absence of a large amount of extraneous protein, as in the case of purified preparations, the virus is immediately and completely inac-

TABLE 6.—*The effect of 0.5 per cent mercuric chloride on virus in untreated juice at various hydrogen-ion concentrations over periods of time up to 3.5 months at 5° C.*

pH	Tested →	At once	After 3 days	After 1 month	After 3.5 months
	Chemical	38.4[a]	60.6	107.5	13.8
3	Chemical ÷ control	79.4[b]	71.4	76.4	33.8
	Chemical	43.5	42.2	96.0	15.9
4	Chemical ÷ control	70.4	72.3	67.2	59.6
	Chemical	33.5	61.5	85.8	8.4
5	Chemical ÷ control	66.5	68.6	50.0	33.6
	Chemical	26.3	55.8	66.6	42.5
6	Chemical ÷ control	68.2	95.0	45.5	64.1
	Chemical	53.6	30.5	81.7	35.2
7	Chemical ÷ control	87.8	66.5	56.2	68.0
	Chemical	52.9	56.7	93.4	22.6
8	Chemical ÷ control	54.3	75.0	50.9	67.0

[a] Numbers represent the average of the actual number of lesions per half-leaf obtained on 10 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with a purified virus preparation containing the designated chemical.

[b] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 10 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the chemical-containing preparation, divided by the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation.

tivated at pH 6, 7, and 8. The protective action which is evidenced at pH 3, 4, and 5 must be due to the hydrogen-ion concentration. This suggests that the virus has an isoelectric point around pH 4.5, and that the protective action is due to the failure of the virus to combine with mercuric chloride at hydrogen-ion concentrations near the isoelectric point of the virus. This explanation appears all the more tenable in view of recent evidence that this virus is a protein (30).

It seemed of interest to determine the effect of large amounts of mercuric chloride on virus in purified preparations and also in untreated juice at pH 4.5. Table 7 gives the results of an experiment in which a 2 per cent solution and a 10 per cent suspension of mercuric chloride at pH 4.5 were added to equal volumes of untreated juice and a purified virus preparation at pH 4.5. Portions of each mixture were dialyzed at once and after standing for different periods of time at 5° C., and tested for infectivity. It may be seen that 1 per cent mercuric chloride had practically no effect on the infectivity of the virus in untreated juice, even after standing for 4 months, and 5 per cent mercuric chloride had only a moderate inactivating action. No bacterial growth was visible in the control preparation at the end of 1 month, although bacterial growth was visible at the end of 4 months. This probably accounts for the low infectivity of the control at the end of the 4-month period. All preparations containing mercuric chloride were without visible bacterial growth. It may also be seen from table 7 that 1 per cent mercuric chloride partially inactivates the virus in purified preparations, and that 5 per cent mercuric chloride almost completely inactivates the virus immediately after the addition of the reagent. It is believed that, although pH 4.5 is sufficiently close to the isoelectric point of the virus to render it inert toward small concentrations of mercuric chloride, it is not close enough to prevent interaction when large amounts of mercuric chloride are present. The action of tobacco-mosaic virus in the presence of mercuric chloride is consistent with the view that the virus is a protein.

Effect of Dipotassium Phosphate on Infectivity

The results of experiments on the effect of 0.1 M dipotassium phosphate on virus infectivity, which are recorded in table 2, indicate that this salt causes only a slight increase when the infectivity is measured on *Nicotiana glutinosa*, but that it causes a marked increase when the infectivity is measured on *Phaseolus vulgaris* variety Early Golden Cluster. Thornberry (32) found that dipotassium phosphate also caused a marked increase in the infectivity of tobacco-mosaic virus when tested on *P. vulgaris* variety Scotia. It seemed desirable to study the nature of the effect of phosphate on infectivity.

The results of an experiment designed to determine whether the effect

TABLE 7.—*The effect of 1 per cent and 5 per cent mercuric chloride on virus in untreated juice and in a purified preparation at pH 4.5 after standing for periods of time up to 4 months at 5° C.*

	Tested →		At once	After 1 week	After 1 month	After 4 months
Untreated juice	1% $HgCl_2$	Chemical	111.8[a]	77.2	73.7	25.2
		Chemical ÷ control	115.0[b]	86.5	98.5	157.0
	5% $HgCl_2$	Chemical	70.7	40.4	67.4	9.6
		Chemical ÷ control	88.4	45.3	37.7	71.0
Purified virus	1% $HgCl_2$	Chemical	57.2	43.0	75.4	13.3
		Chemical ÷ control	45.5	66.9	84.3	42.3
	5% $HgCl_2$	Chemical	2.7	0.0	0.0	0.0
		Chemical ÷ control	2.0	0.0	0.0	0.0

[a] Numbers represent the average of the actual number of lesions per half-leaf obtained on 10 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with a purified virus preparation containing the designated chemical.

[b] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 10 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the chemical-containing preparation, divided by the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation.

of phosphate is due to the hydrogen-ion concentration changes that it causes or to some effect of the phosphate in itself are given in table 8. Portions of juice from diseased Turkish tobacco plants grown in a field were adjusted to different hydrogen-ion concentrations by the addition of equal portions of 0.1 M solutions of various buffers. In some cases it was necessary to add small amounts of 2 N HCl or NaOH in order to attain the desired hydrogen-ion concentrations. The solutions at the various hydrogen-ion concentrations were then tested for infectivity by the half-leaf method on plants of *Phaseolus vulgaris* and *Nicotiana glutinosa*. It may be seen from table 8 that, regardless of the buffer used, high hydrogen-ion concentrations cause a decrease in infectivity, which, as has already been pointed out, is considerable in the case of *P. vulgaris*, and low hydrogen-ion concentrations, within certain limits, tend to cause an increase in infectivity, which is also more marked in the case of *P. vulgaris*. At pH 5, 6, 7 and 8 the greatest increase in infectivity as measured on *P. vulgaris* occurred with

preparations containing phosphate. With one exception this was true when the infectivity was measured on *N. glutinosa*, although the increase was not nearly so marked as it was with *P. vulgaris*. There is some indication, therefore, that part of the increased infectivity in the presence of dipotassium phosphate is due to some action on the virus and part due to some action on the test plant.

TABLE 8.—*Effect of various buffers on the infectivity of tobacco-mosaic virus*

pH	Buffers	Phaseolus vulgaris	Nicotiana glutinosa
1	Phosphoric + hydrochloric acid	0.0[a]	0.0[a]
1	Hydrochloric acid	0.0	0.0
2	Hydrochloric acid	1.5	6.4
3	Citric acid	1.0	9.4
4	Sodium citrate + hydrochloric acid	4.4	7.2
4	Potassium phosphate + citric acid	3.8	8.2
4	Potassium acid phthalate	5.2	5.3
5	Sodium citrate + sodium hydroxide	11.6	10.2
5	Potassium phosphate + citric acid	19.7	12.8
5	Potassium acid phthalate + sodium hydroxide	12.5	14.7
5.5	Untreated diseased juice	35.9	10.4
6	Potassium acid phthalate + sodium hydroxide	20.5	10.3
6	Potassium phosphate + citric acid	73.5	26.1
6	Potassium phosphate	33.9	33.5
7	Potassium phosphate	50.1	31.0
7	Sodium veronal + hydrochloric acid	24.3	16.6
8	Sodium veronal + hydrochloric acid	59.3	28.5
8	Potassium phosphate	79.7	49.8
8	Sodium borate + sodium hydroxide	37.8	44.1
9	Sodium borate + sodium hydroxide	67.4	15.6
10	Sodium borate + sodium hydroxide	29.6	4.0

[a] Numbers represent the average of the actual number of lesions per half-leaf obtained on 20 or more half-leaves of *Phaseolus vulgaris* or 10 or more half-leaves of *Nicotiana glutinosa* on inoculation with the designated preparation.

An experiment was made for the purpose of determining the effect of different inoculation methods on the increase in infectivity caused by 0.1 M dipotassium phosphate and charcoal. Juice from diseased Turkish tobacco plants grown in a greenhouse was diluted with water and to portions of each dilution was added 0.1 gm. of charcoal (norite) per 10 cc. of the diluted juice. Another portion of juice was made up to 0.1 M phosphate by the addition of solid salt and then diluted with 0.1 M phosphate at pH 7. Plants of *Nicotiana glutinosa* were then inoculated with dilutions of the three preparations by rubbing and by needle puncture. It may be seen from table 9 that charcoal caused a marked increase in infectivity at all

TABLE 9.—*Effect of needle-puncture and rubbing methods of inoculation on the infectivity of tobacco-mosaic virus in the presence of dipotassium phosphate and charcoal*

Dilution	Preparation number	Rubbing inoculation			Needle-puncture inoculation		
		Untreated juice	Untreated juice + 1% charcoal	Untreated juice + 0.1 M phosphate	Untreated juice	Untreated juice + 1% charcoal	Untreated juice + 0.1 M phosphate
1:3	1	156.5_R[a] 203.5_L[a] 97.0_R 105.9_L[a] 119.6_L 211.9_R			0.96_R[b] 2.32_L[b] 0.91_L		0.51_R[b]
	2	101.5_L		99.5_R	1.52_R 1.56_L	1.7_L	1.12_R
	3	135.1_L 197.2_R 157.5_L 190.0_R			5.48_L 4.5_R	6.2_L	5.5_R
10^{-2}	1	63.4_R 167.9_L 63.4_R 53.8_L 22.5_R 20.4_L 27.2_L 67.5_R			0.48_R 0.6_L	0.96_L	0.48_R
	2	101.1_R		140.9_L	0.8_L 1.16_R	0.52_R	0.7_L
	3	99.0_L 230.9_R 121.5_L 155.2_R			1.8_R 2.1_R	2.1_L	2.4_L
10^{-3}	1	25.8_R 76.0_L 15.0_R 11.7_L 7.6_R 3.5_L 11.8_L 118.0_R					
	2	84.4_L		92.7_R			
	3	57.6_L 124.8_R 65.5_R 103.8_L					
10^{-4}	1	18.4_R	54.3_L				
	2	58.0_R		82.6_L			
	3	33.3_R 75.9_L 20.5_L 50.8_R					
10^{-5}	1	1.7_R	9.9_L				
	2	3.5_L		3.6_R			
	3	5.8_L 9.1_R 6.6_R 11.1_L					

[a] Numbers represent the average of the actual number of lesions per half-leaf obtained on 10 half-leaves of *Nicotiana glutinosa* on inoculation by rubbing of the designated preparation.

[b] Numbers represent the average of the actual number of lesions per half-leaf obtained on 50 leaves of *N. glutinosa* on inoculation of the designated preparation. Inoculation by needle puncture, 100 punctures per half-leaf.

R indicates inoculation was on right leaf-halves.

L indicates inoculation was on left leaf-halves.

dilutions with both methods of inoculation. Phosphate, however, caused either no change or a slight decrease in infectivity when the needle-puncture method of inoculation was used, and no appreciable change at low dilutions but an increase in infectivity at high dilutions when the rubbing method of inoculation was used. The experiment proves that phosphate does not cause an increase in infectivity under all conditions. Further demonstration of this fact was afforded in an experiment in which dilutions of untreated juice in 0.1 M phosphate at pH 7 in the presence and absence of charcoal were used to inoculate small Turkish tobacco plants. Dilutions of the juice with water were also tested in the presence and absence of charcoal. Each dilution of each preparation was inoculated to 50 small Turkish tobacco plants by rubbing the preparation once over one leaf of each plant. The results of the experiment, which are given in table 10, show that 0.1 M phosphate at pH 7 caused a decrease in infectivity at high dilutions, but that charcoal, even in the presence of phosphate, caused a marked increase in infectivity. Similar results were obtained in an experiment in which the inoculation was carried out by making 5 needle punctures on one leaf of each plant. As a whole, the results show that phosphate may have a variable effect on infectivity, depending on the dilution of the mosaic-diseased juice and on the plant used to measure infectivity. It may cause a decrease in infectivity in the case of Turkish tobacco plants, a decrease, no change, or an increase in the case of *N. glutinosa* plants, and a marked increase in infectivity in the case of *Phaseolus vulgaris* plants.

TABLE 10.—*Effect of phosphate and charcoal on the infectivity of tobacco-mosaic virus as measured on Turkish tobacco plants*

Dilution	Diseased juice diluted with water	Diseased juice diluted with water + 1% charcoal	Diseased juice diluted with 0.1 M phosphate at pH 7	Diseased juice diluted with 0.1 M phosphate at pH 7 + 1% charcoal
10^{-2}	48[a]	48	48	48
10^{-3}	48	48	45	48
10^{-4}	34	47	21	46
10^{-5}	7	32	1	34
10^{-6}	5	9	2	15

[a] Numbers represent the number of diseased plants out of 48 inoculated. Preparations rubbed once over one leaf of each plant.

Effect of Charcoal on Infectivity

Vinson and Petre (33) reported that charcoal caused an increase in the infectivity of tobacco-mosaic virus. Similar results were obtained in experiments in which virus was tested in the presence of phosphate and charcoal, the results of which are given in tables 9 and 10. Since little is known concerning the cause of this effect, it seemed desirable to study the action of charcoal in more detail. Table 11 records the results of an experiment in which a purified virus preparation was diluted with 0.1 M phosphate at pH 7 and various amounts of charcoal (Nuchar 00) were added to portions of each of the dilutions before inoculation to plants of *Phaseolus vulgaris*. It may be seen that from 0.01 per cent to 1 per cent charcoal by weight caused an increase in the number of lesions at all dilutions tested, with but 3 exceptions. However, 5 per cent charcoal still caused a marked increase in the number of lesions at a 1:10 dilution of virus, but at higher dilutions it caused a decrease in number of lesions, even preventing infection at the 1:100,000 dilution. Large amounts of charcoal caused a reduction in the number of lesions, and always prevented infection at high dilutions. Thus the effect of charcoal on infectivity may also be quite variable, causing an increase, no change, or a decrease in infectivity, depending on the relative proportions of virus and charcoal in the inoculum.

TABLE 11.—*Effect of different amounts of charcoal on the infectivity of a purified preparation of tobacco-mosaic virus*

Dilution of virus[a] →	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Virus	473[b]	234	81	11	2
Virus + 0.01% Nuchar 00	790	335	214	24	3
" + 0.1% " "	1052	381	296	33	1
" + 0.5% " "	1280	556	338	49	3
" + 1.0% " "	1457	735	215	6	2
" + 5.0% " "	1460	227	14	0.5	0
" + 10% " "	435	27	2	0	0
" + 20% " "	83	15	1	0	0

[a] Dilutions were made with 0.1 M phosphate at pH 7.

[b] Numbers represent the average of the actual number of lesions per leaf obtained on 14 leaves of *Phaseolus vulgaris* on inoculation with the designated preparation.

It appeared likely that a decrease in infectivity caused by large amounts of charcoal might be due to adsorption of virus on particles of charcoal. Several experiments concerned with the adsorption of virus on different

TABLE 12.—*Adsorption of tobacco-mosaic virus on charcoal*

	Phaseolus vulgaris				Nicotiana glutinosa			
Dilution[a] →	1	10	100	1000	1	10	100	1000
Virus (control) pH 6.7	157.5[c]	117.0	99.0	66.5	196.0[c]	173.1	104.3	31.0
Filtrate from (virus at pH 3 + 10% Nuchar 00[b])	105.0	59.4	11.3	4.9	31.8	71.8	9.6	2.3
" " " " pH 5 " " " "	98.0	95.4	38.8	3.9	100.5	96.4	10.9	1.5
" " " " pH 6.7 " " " "	144.5	127.6	91.4	36.2	238.0	165.5	92.9	19.3
" " " " pH 8.1 " " " "	130.5	116.7	55.5	16.2	141.1	87.4	42.1	9.7
" " " " pH 9.2 " " " "	87.1	69.0	54.5	5.5	183.4	58.5	48.0	11.0
" " " + 10% Nuchar XXX)	93.8	14.3	2.2	0.4	52.3	1.0	1.7	0.2
" " " " " " No. 2)	99.0	69.3	29.5	9.4	88.5	56.7	24.8	5.8
" " " " " " W)	127.1	97.0	57.2	9.3	113.2	121.8	19.0	14.4
" " " " " Norite)	159.9	146.0	100.9	17.5	161.2	130.0	49.0	17.5
" " " " " Nuchar 00)	144.5	127.6	91.4	36.4	238.0	165.5	92.9	19.3
" " (above " " "	3.0	0.7	0.4	0.2	3.0	0.3	0.3	0.0
" " " " " "	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Virus (control) pH 7.0	436.0[c]	394.0	205.0	71.5				
Filtrate from (virus + 10% Nuchar 00)	218.0	123.0	71.7	1.1				
" " (above " " "	1.8	0.0	0.0	0.0				
" " " " " "	1.1	0.0	0.0	0.0				
" " (virus + 5% " "	474.0	347.0	204.0	105.0				
" " (above " " "	72.3	27.2	3.9	0.8				
" " " " " "	1.4	0.2	0.0	0.0				
" " " " " "	0.0	0.0	0.0	0.0				

[a] Dilutions were made and then the charcoal was added.

[b] Preparations adjusted to pH 6.7 before inoculation to plants.

[c] Numbers represent the average of the actual number of lesions per leaf obtained on 12 or more leaves of *Phaseolus vulgaris* or 5 or more leaves of *Nicotiana glutinosa* on inoculation with the designated preparation and dilution.

kinds of charcoal under many different conditions were made. Purified and fairly concentrated virus preparations were used in most of these experiments. Unless otherwise specified, the dilutions were made with 0.1 M phosphate at pH 7 and the designated amount of charcoal was then added to each of the dilutions of the virus preparation. After stirring for 10 minutes, the mixtures were filtered through hardened filter paper. The amount of adsorption of virus on charcoal was judged by the infectivity of the filtrate. It may be seen from table 12 that adsorption of the virus on charcoal is at a maximum around pH 3 to 5, although some virus is adsorbed at all reactions tested. The results also indicate that more virus is adsorbed by the more finely divided charcoal preparations, such as Nuchar XXX, than by the coarser grades of charcoal. It may also be seen from table 12 that two treatments with 10 per cent by weight of charcoal, or 3 treatments with 5 per cent charcoal remove all of the virus from even fairly concentrated preparations. The results given in the first 4 rows of table 13 demonstrate that even 0.01 per cent to 0.5 per cent charcoal adsorbs a measurable amount of virus. The increase in the number of lesions at low dilutions of virus is probably due to the effect of small particles of charcoal that pass the filter paper. A striking demonstration of the effects that the same amount of charcoal may have on different amounts of virus is given in the 5th and 6th rows of table 13. When 1 per cent charcoal is added to a fairly concentrated virus preparation, the relative amount of virus adsorbed and removed on the charcoal on filtration is very small, so that the numbers of lesions produced before and after filtration are about the same. At a 1 : 10 dilution of virus, however, it may be seen that the filtrate from the 1 per cent charcoal gives only about one-half as many lesions as the preparation gave before filtration. At a 1 : 1000 dilution of the virus the adsorption and removal of the virus on the charcoal caused a reduction in lesions from 280 to 41, indicating that much of the virus had been removed on the charcoal. Thus, the net effect on the infectivity may vary widely, depending on the ratio between amount of virus and amount of charcoal. It may also be seen from table 13 that the first treatment with 10 per cent charcoal removed practically all of the virus, since, when this charcoal was resuspended and then diluted and tested, it gave many lesions even at a 1 : 10,000 dilution, whereas dilutions of the suspension of the second portion of charcoal gave only a few lesions. That virus in untreated mosaic-diseased juice may also be adsorbed and removed on charcoal is indicated by the results given in the last 3 rows of table 13.

DISCUSSION

Tests of the effect of a large number of chemicals on the infectivity of tobacco-mosaic virus have demonstrated that, with the exception of brucine, the only reagents that affect this virus directly are oxidizing agents, pro-

TABLE 13.—*Adsorption of tobacco-mosaic virus on various amounts of charcoal*

Dilution[a] →	1	10	100	1000	10,000	100,000
Virus (control) pH 7.0	889[e]	526	398	204	88.3	29.8
Filtrate from (virus + 0.01% Nuchar 00)		950	407	312	71.0	12.2
" " " + 0.1 % " "		991	466	129	14.2	3.4
" " " + 0.5 % " "		960	210	45	6.5	1.4
Virus + 1% Nuchar 00[b]	1448	1950	853	280	11.9	1.8
Filtrate from (virus + 1% Nuchar 00)	1367	1060	121	41	2.3	0.7
" " " + 5% " "		67	0.3	0.9	0.0	0.0
" " " + 10% " "	452	0.1	0.0	0.0	0.0	0.0
" " (above " " "	0.3	0.2	0.0	0.0		
Suspension of first 10% Nuchar 00 in 10 volumes 0.1M phosphate pH 7	2320	2310	673	373	76.4	
Suspension of second 10% Nuchar 00 in 10 volumes 0.1M phosphate pH 7	4.0	3.9	1.1	1.2	1.0	
Virus + 10% Nuchar 00[c]	602	374	167	24.9		
Untreated diseased juice[d]	63.4	26.2	2.4	0.4		
Filtrate from (above + 10% Nuchar 00[d])	2.0	0.2	0.0	0.0		
" " " " " "	0.0	0.0	0.0	0.0		

[a] Dilutions were made and then the charcoal was added.

[b] Charcoal not removed by filtration.

[c] Charcoal added and then dilutions were made. No additional charcoal used.

[d] Dilutions were made with water.

[e] Numbers represent the average of the actual number of lesions per leaf obtained on 12 or more leaves of *Phaseolus vulgaris* on inoculation with the designated preparation and dilution.

tein-precipitating agents, and materials causing a hydrogen-ion concentration known to inactivate virus. It has not been found possible to reverse the inactivation caused by oxidation by subsequent treatment with reducing agents, although inactivation caused by potassium permanganate may be interrupted by the addition of cysteine. However, the exact nature of the oxidation reaction has not been determined. The fact that this virus is precipitated by so many protein-precipitating agents is an indication that it is protein in nature. The action of salts of heavy metals, particularly mercuric chloride, is very significant. Proteins are relatively inert chemically at or near their isoelectric point, and are more reactive at hydrogen-ion concentrations removed from the isoelectric point. Mercuric chloride was found to affect virus to a markedly less extent at pH 3, 4, and 5 than at pH 6, 7, and 8. This suggests that this virus may be a protein having an isoelectric point near pH 4.5, thus rendering it inactive chemically toward mercuric chloride near pH 4.5, but allowing reaction with mercuric chloride at pH 6, 7, or 8. The fact that tobacco-mosaic virus was found to be unaffected over long periods of time by concentrations of mercuric chloride known (7, 23, 31) to be germicidal, indicates that it is not a bacterial organism. As a whole, the results are in harmony with the conception that this virus is a protein.

SUMMARY

The effect of 110 chemicals on the infectivity of purified preparations of tobacco-mosaic virus has been determined. Many chemicals were found that affected neither the virus nor the test plant. A few chemicals caused an increase in infectivity of virus. Many chemicals were found that caused a marked decrease in the infectivity of virus.

The necessity of differentiating between the action of chemical on virus and the action of chemical on the plant used to determine the infectivity of virus has been demonstrated.

In general, the chemicals that had a direct inactivating action on tobacco-mosaic virus may be classified as oxidizing agents, protein-precipitating agents, and agents causing a hydrogen-ion concentration known to inactivate the virus.

The fact that tobacco-mosaic virus was found to be unaffected over long periods of time by concentrations of mercuric chloride known to be germicidal is an indication that the virus is not a bacterial organism. Virus in purified preparations was affected by mercuric chloride at pH 6, 7, and 8 to a markedly greater extent than at pH 3, 4, and 5. These results are in accord with recent evidence that this virus is a protein.

Dipotassium phosphate or phosphate buffers near pH 7 cause a marked increase in the infectivity of tobacco-mosaic virus when tested on *Phaseolus*

vulgaris, but when tested on Turkish tobacco plants may cause a reduction in infectivity, and when tested on *Nicotiana glutinosa* may cause no change, an increase, or a decrease in infectivity.

Charcoal may cause no change, an increase, or a decrease in the infectivity of tobacco-mosaic virus, depending upon the ratio of amount of charcoal to amount of virus. Tobacco-mosaic virus may be completely removed from solution by adsorption on charcoal. Maximum adsorption occurs at pH 3 to 5 and with very finely divided charcoal.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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CHEMICAL STUDIES ON THE VIRUS OF TOBACCO MOSAIC

V. DETERMINATION OF OPTIMUM HYDROGEN-ION CONCENTRATIONS FOR PURIFICATION BY PRECIPITATION WITH LEAD ACETATE[1]

W. M. STANLEY

(Accepted for publication August 5, 1935)

Vinson and Petre (6), in 1929, found that the addition of small amounts of basic lead acetate to the juice of mosaic-diseased Turkish tobacco plants caused the precipitation of most of the pigment and protein without apparently removing or injuring the tobacco-mosaic virus. Two years later Vinson and Petre (7) outlined a procedure for obtaining virus from juice of diseased plants by precipitation with neutral lead acetate following a preliminary treatment with basic lead acetate to remove pigment. They reported that their preparations, which contained only about 1 per cent of the original solids content of the juice, had an infective power equal to that of the fresh juice, although they found that the discarded basic lead acetate precipitate contained as much as $\frac{1}{3}$ of the virus. Apparently they did not attempt to prevent the loss of virus during the basic-lead-acetate treatment. Because the colorless water-clear solutions which Vinson and Petre obtained with the lead-acetate method were indisputably very infectious and thus useful in many ways, and because of the possibility of improving the process in at least one of the steps, it was decided to make a detailed study of the purification of tobacco-mosaic virus with lead acetate. The present paper records the results of this study.

MATERIALS AND METHODS

The virus preparations used in the experiments to be reported consisted of the untreated juice from plants of mosaic-diseased *Nicotiana tabacum* L. variety Turkish grown in a field during the summer months. The plants were cut and kept in a freezing room for a few days and then allowed to thaw just before pressing. The juice was then filtered through 4 layers of bandage gauze.

Merck's reagent lead subacetate and neutral lead acetate were used for the preliminary precipitation of pigment and the precipitation of virus, respectively. Preparations containing 200 gm. of reagent per liter of solution were used. Determinations of hydrogen-ion concentrations were made by

[1] Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by date of receipt of the manuscript.

means of a MacInnes type glass electrode (2). Tests for virus infectivity were made on *Nicotiana glutinosa* L. and *Phaseolus vulgaris* L. variety Early Golden Cluster by the half-leaf method of inoculation previously described (1, 3, 4). It was found necessary, because of the variable effect of phosphate on virus infectivity (5), to dilute untreated diseased juice and the treated preparations with the same diluent whenever tests for infectivity were made. Unless otherwise specified, 0.1 M phosphate at pH 7 was used as a diluent, although in some instances, as a check, the phosphate was removed from the treated preparations by dialysis and then water was used as a diluent for these preparations and the untreated juice.

The 3 principal steps involved in the lead-acetate process consist of a preliminary precipitation with lead subacetate, the precipitation of virus with neutral lead acetate, and the elution of the virus from the latter precipitate. Preliminary experiments indicated that the amounts of lead subacetate and neutral lead acetate suggested by Vinson and Petre represented optimum quantities for average juice preparations. However, for preparations containing more than an average amount of protein, it was found that the relative amounts of these reagents should be increased, and for preparations containing less than an average amount of protein, it was found that the amounts should be decreased. The experiments in the present investigation were, therefore, confined to the determination of the optimum hydrogen-ion concentrations for carrying out each of the 3 principal steps in the process. This was done by 3 types of experiments. In the first the lead subacetate precipitation was carried out at various hydrogen-ion concentrations using separate portions of the same infectious juice preparation. The subsequent treatment of each of the portions was the same. In the second set of experiments the lead subacetate precipitation was carried out at the optimum hydrogen-ion concentration, as indicated by the first experiment. The preparation was then divided into several portions and the neutral lead acetate precipitation made at various hydrogen-ion concentrations. The subsequent treatment of each of the portions was identical. In the third experiment the two lead acetate precipitations were carried out at the optimum hydrogen-ion concentrations, as determined in the first two sets of experiments. A suspension of the neutral lead acetate precipitate was made and divided into several equal portions and these portions adjusted to various hydrogen-ion concentrations for elution of virus. The infectivities of the final preparations in each of the experiments were compared with each other.

EXPERIMENTAL

Four 200 cc. portions of untreated infectious juice were adjusted to pH 6, 7, 8, and 9, respectively, by the addition, with stirring, of from 2 drops to 2 cc. of 2 N NaOH. Then 6 cc. of a solution of lead subacetate containing

TABLE 1.—*Effect of various hydrogen-ion concentrations on the lead subacetate precipitation*

Dilution →		1		10		100		1000	
pH of precipitation		Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris	Nicotiana glutinosa
6	L[b]	10.8[c]	31.7[c]	12.4	43.4	2.0	12.0	1.2	6.0
7	R[b]	24.1	64.9	10.5	40.2	2.3	15.5	1.1	5.3
7	L	27.4	60.5	9.9	23.7	4.4	15.7	0.9	4.6
8	R	59.9	107.1	28.1	30.2	7.5	10.2	1.9	6.9
8	L	28.8	85.4	27.3	34.0	8.0	17.5	1.9	5.3
9	R	67.4	101.8	74.6	49.4	21.3	26.4	3.8	7.7
9	L	74.0	63.4	57.7	58.4	27.0	19.3	2.9	12.0
×[a]	R	65.3	60.6	48.7	47.6	24.1	17.4	6.6	18.5
×[a]	L	80.0	55.4	47.8	60.3	17.6	46.0	6.9	12.7
6	R	36.2	22.0	11.0	30.0	4.7	13.2	2.4	4.0

[a] The lead subacetate precipitation was omitted. Subsequent treatment same as other preparations.

[b] L and R indicate that inoculation was on left and right half-leaves, respectively.

[c] Numbers represent the average of the actual number of lesions on 10 or more half-leaves of *Phaseolus vulgaris* or 5 half-leaves of *Nicotiana glutinosa* on inoculation with the designated preparation and dilution.

200 gm. of reagent per 1 liter of solution was added, with stirring, to each preparation. The preparations were then centrifuged for 10 minutes and the supernatant liquids adjusted to pH 4.6 by the addition of from 1.7 to 3 cc. of 2 N HCl. A 5th 200 cc. portion of infectious juice was adjusted to pH 4.6 by the addition of 1.1 cc. of 2 N HCl and then 14 cc. portions of a preparation of 200 gm. of neutral lead acetate in 1 liter of solution were added to this, and to the other 4 preparations. After being stirred for 15 minutes, the 5 preparations were centrifuged for 10 minutes and the supernatant liquids from the cream-colored precipitates were discarded. Each precipitate was washed twice with 150 cc. of 0.3 M KH_2PO_4 and then suspended in 60 cc. of 0.1 M phosphate at pH 6.8 and stirred for 1 hour. The 5 mixtures were frozen overnight, then thawed, centrifuged and the supernatant liquids tested for virus activity. The results of the tests, which are given in table 1, show that much virus is lost when the lead subacetate precipitation is carried out at pH 6 or 7, and that but little virus is lost when the precipitation is carried out at pH 9. Precipitation at pH 9 is definitely superior to precipitation at pH 8. A comparison of the lesion counts for the preparations precipitated at pH 6 and 9 with those for the preparation in which the lead subacetate precipitation was omitted indicates that about 90 per cent of the virus is lost at pH 6, whereas the loss at pH 9 is less than 10 per cent. If, therefore, the procedure outlined by Vinson and Petre is modified to the extent that the lead subacetate precipitation is carried out at about pH 9 instead of at pH 6.5 as they recommend, a marked increase in the yield of virus may be obtained.

The effect of different hydrogen-ion concentrations on the neutral lead acetate precipitation was determined by adjusting 800 cc. of untreated infectious juice to pH 9.1 by adding 10 cc. of 2.3 N NaOH with stirring. Then 24 cc. of lead subacetate solution was added and, after stirring for 5 minutes, the preparation was centrifuged for 10 minutes and the supernatant liquid, now at pH 8.7, was divided into 4 equal portions. These were adjusted to pH 3, 4, 5, and 6 by the addition of 10, 5, 2.3, and 1.4 cc., respectively, of 2 N HCl, and then 14 cc. of neutral lead acetate solution was added to each, with stirring. After 15 minutes the 4 preparations were centrifuged and the supernatant liquids which were at pH 3.8, 4.6, 5.2, and 5.5, respectively, were discarded. The subsequent treatment of the 4 precipitates, involving washing with KH_2PO_4, freezing and elution with phosphate at pH 6.8, was exactly the same as that described in the first experiment. The 4 preparations were tested on leaf-halves against each other and also against a portion of the untreated infectious juice used as starting material. The results, which are given in table 2, indicate that the yield of virus is about the same whether the neutral lead acetate precipitation be carried out at pH 3, 4, 5, or 6. There is some slight indication that precipitation at pH 5 or 6 is pref-

TABLE 2.—*Effect of various hydrogen-ion concentrations on the neutral lead acetate precipitation of virus*

Dilution →		1		10		100		1000	
pH of precipitation		Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris	Nicotiana glutinosa
3	L[b]	81.5[c]	59.4[c]	30.7	59.2	13.2	27.2	2.4	13.4
4	R[b]	131.0	79.4	60.5	63.6	13.1	38.8	2.7	13.0
4	L	95.1	70.0	85.3	68.8	18.3	11.8	3.4	8.0
U.J.[a]	R	13.7	33.8	60.4	40.2	46.8	12.0	12.2	8.8
4	L	107.4	88.8	87.1	83.4	14.3	31.0	5.1	9.0
5	R	147.9	107.6	135.0	75.2	33.8	29.2	9.2	7.8
5	L	158.6	75.6	89.6	68.6	21.9	27.0	3.6	7.6
6	R	112.7	84.8	68.1	71.0	23.1	21.6	5.7	8.0
6	L	111.2	83.6	74.3	60.8	9.7	11.8	3.2	12.6
3	R	104.9	95.6	70.3	68.8	10.7	19.2	1.7	10.6

[a] Untreated infectious juice used for starting material. No chemical treatment.

[b] L and R indicate that inoculation was on left and right half-leaves, respectively.

[c] Numbers represent the average of the actual number of lesions obtained on 10 or more half-leaves of *Phaseolus vulgaris* or 5 half-leaves of *Nicotiana glutinosa* on inoculation with the designated preparation and dilution.

erable to precipitation at pH 3 or 4, so for routine preparations of virus the neutral lead acetate precipitation has been carried out at about pH 5.5.

The effect of different hydrogen-ion concentrations on the elution of virus from the neutral lead acetate precipitate was next studied. Twelve liters of untreated infectious juice was adjusted to pH 9.1 by the addition of 160 cc. of 2 N NaOH and then 360 cc. of lead subacetate solution was added slowly, with stirring. The mixture which was at pH 8.9 was centrifuged and the supernatant liquid adjusted to pH 5.5 by the addition of 120 cc. of 2 N HCl. Then 840 cc. of neutral lead acetate solution was added with stirring and, after standing for 30 minutes, the preparation was centrifuged and washed twice with 2 liters and once with 1 liter of 0.33 M KH_2PO_4. The precipitate was then evenly suspended in 1.2 liters of 0.1 M phosphate at pH 6.8 and divided into 6 portions of 200 cc. each. These portions were then adjusted to pH 4, 5, 6, 7, 8, and 9 by the addition of 6, 5.3, and 1.5 cc. of 2 N HCl and 1.5, 6.3, and 9 cc. of 2 N NaOH, respectively, and then frozen overnight. The next day the 6 preparations were thawed, centrifuged, the supernatant liquids removed, all adjusted to pH 7, and tested for infectivity. The results, which are presented in table 3, show that the optimum hydrogen-ion concentration for elution of virus from the neutral lead acetate precipitate is pH 7. A decreased yield of virus is obtained at pH 6, 8 and 9, and practically no virus is removed at pH 4 and 5. The optimum hydrogen-ion concentrations for carrying out the lead acetate process for purifying virus are, therefore, about pH 9 for the lead subacetate precipitation, about pH 5.5 for the neutral lead acetate precipitation, and about pH 7 for the elution of virus.

DISCUSSION

A greatly increased yield of virus may be obtained by modifying slightly the lead-acetate process for virus purification outlined by Vinson and Petre, by carrying out the lead subacetate precipitation at about pH 9 instead of at pH 6.5. Practically colorless, water-clear solutions of virus which are almost as infectious as the starting material may be obtained by this modified process. Virus preparations more infectious than the starting material may be obtained by reducing the volume of the phosphate eluent to less than that of the volume of the starting material. For example, preparations about 10 times as infectious as the starting material were obtained by reducing the volume of the eluent to 1/20 of that of the starting material. After such concentration, however, the preparations were always quite colored and also had a high solids content. Because of this, the modified lead-acetate process has not been found useful in preparing highly concentrated virus preparations. However, it has much to commend it as a method for preparing rap-

TABLE 3.—*Effect of various hydrogen-ion concentrations on elution of virus from the neutral lead acetate precipitate*

Dilution →		1		10		100		1000
pH of eluent		Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris
4	L[a]	1.3[b]	1.2[b]	0.4	0.0	0.0	0.0	0.0
5	R[a]	22.5	7.3	13.9	3.0	1.3	0.1	0.1
5	L	4.5	125.1	4.5	1.1	0.2	0.7	0.1
6	R	5.3	51.8	103.8	16.4	28.6	14.0	9.7
6	L	51.4	29.3	62.8	11.6	30.1	6.4	4.1
7	R	110.0	25.4	71.0	18.8	43.9	8.0	10.9
7	L	135.5	25.0	38.0	12.3	44.8	5.0	11.9
8	R	76.5	35.6	33.7	12.5	39.0	6.6	9.0
8	L	208.0	29.4	88.4	15.6	45.7	9.9	9.5
9	R	171.0	35.0	44.2	14.6	16.5	7.2	4.8
9	L	107.5	43.4	52.8	29.0	44.7	9.1	5.1
4	R	0.8	1.5	0.8	0.0	0.1	0.0	0.0

[a] L and R indicate that inoculation was on left and right half-leaves, respectively.

[b] Numbers represent the average of the actual number of lesions obtained on 16 or more half-leaves of *Phaseolus vulgaris* or 5 or more half-leaves of *Nicotiana glutinosa* on inoculation with the designated preparation and dilution.

idly small or even reasonably large amounts of fairly pure and moderately concentrated virus.

The modified process has been used routinely for the preparation of virus solutions used in the experiments reported in previous papers of this series. Infectivity tests on the partially purified preparations have demonstrated that a final yield of from 50 to 90 per cent of the virus in the starting material may be expected with the modified process. Experiments herein reported indicate that the original process outlined by Vinson and Petre results in a loss of about 90 per cent of the virus in the original juice. The greatest loss of virus occurs on the two lead acetate precipitates. Suspensions of the lead subacetate precipitates were always found to be infectious, although with the modified process the activity diminished rapidly on dilution, a 1:1000 dilution usually being noninfectious. The loss on the neutral lead acetate precipitate is due to incomplete elution and can be partially avoided by successive elutions of the precipitate. Many tests on the dialyzed supernatant from the neutral lead acetate precipitation demonstrated that it was, in most instances, practically, if not entirely, free of virus. Tests on the dialyzed KH_2PO_4 washes from the neutral lead acetate precipitate indicated that they contained either no virus or a very small amount of virus.

For routine use of the method, it is necessary that the starting material contain near a normal amount (2 to 4 per cent) of total solids. If a starting material is used that has been frozen, thawed and centrifuged several times and that has, as a result, a solids content of only about 1 per cent, then the lead subacetate precipitation will bring down most of the virus protein and the final preparation will contain practically no virus. If, on the other hand, the starting material contains 10 or 15 per cent total solids, the lead subacetate will fail to precipitate the pigment and the final preparation will contain a much greater percentage of inactive protein and will also be colored.

SUMMARY

The optimum hydrogen-ion concentrations for carrying out the 3 principal steps in the lead acetate process for the purification of tobacco-mosaic virus proposed by Vinson and Petre have been determined. The optimum hydrogen-ion concentration for the lead subacetate precipitation was found to be about pH 9, for the neutral lead acetate precipitation about pH 5.5, and for the elution of the virus from the neutral lead acetate precipitate about pH 7. A greatly increased yield of virus may be obtained by modifying their process slightly and carrying out the lead subacetate precipitation at about pH 9 instead of at pH 6.5. The modified process was found very useful for the rapid preparation of colorless partially purified solutions hav-

ing a virus concentration equal to or somewhat greater than that of the starting material.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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EFFECT OF TANNIC ACID ON THE INFECTIVITY OF TOBACCO-MOSAIC VIRUS

H. H. THORNBERRY

(Accepted for publication May 4, 1935)[1]

Infectivity of tobacco-mosaic virus has been reported to be destroyed by certain chemicals and juices from certain plants. Whether destruction of infectivity resulted from inactivation of virus, from some effect on the host plant, or both, is not apparent. According to Stanley,[2] active trypsin prevents tobacco-mosaic virus infection by rendering the host plant non-susceptible. This protective action against infection occurred when trypsin was applied to the plants before or within 30 minutes after inoculation, but failed when the activity of the enzyme had been destroyed by the action of pepsin or by heat. Allard[3] reported that infectivity of tobacco-mosaic virus was destroyed in 5 days by tannic acid at a concentration of 1 part to 20 or 50 parts of juice and that it was almost destroyed in the same time by a concentration of 1 part of acid to 100 or 200 parts of juice. Unpublished data from previous experiments indicated that infectivity of virus at a 1×10^{-2} dilution, when measured by the local-lesion method on Scotia beans, was inhibited in 1 hour by a 1 per cent solution of tannic acid. Olitsky and Cox[4] reported that tannic acid prevented equine encephalomyelitis virus infection in mice when the acid was applied previous to intranasal inoculation, but had no effect on activity of the virus when applied 15 minutes after inoculation. The purpose of this paper is to report results obtained from experiments that were designed to show the effect of tannic acid on the infectivity of tobacco-mosaic virus.

EXPERIMENTATION

EFFECT OF TANNIC ACID ON BUFFERED VIRUS

Tobacco-mosaic virus at a 1×10^{-2} dilution in 0.1 molar phosphate buffers at reactions of pH 10.0, pH 8.5, pH 7.0, pH 4.5, and pH 3.0 was treated with tannic acid[5] at different concentrations. At the end of the desired exposure

[1] Published at the expense of The Rockefeller Institute for Medical Research out of the order determined by date of receipt of the manuscript.

[2] Stanley, W. M. Chemical studies on the virus of tobacco mosaic. I. Some effects of trypsin. Phytopath. 24: 1055–1085. 1934.

[3] Allard, H. A. Effects of various salts, acids, germicides, etc., upon the infectivity of the virus causing the mosaic disease of tobacco. Jour. Agr. Res. 13: 619–637. 1918.

[4] Olitsky, P. K., and H. R. Cox. Temporary prevention by chemical means of intranasal infection of mice with equine encephalomyelitis virus. Science n.s. 80: 566–567. 1934.

[5] Tannic acid used was Mallinckrodt's di-gallic acid c. p.

of virus to acid, infectivity was measured by rubbing the suspension on the primary leaves of 20 Scotia bean plants. Control plants were inoculated with the virus diluted to the same extent in the buffers. The number of local lesions produced from the various suspensions is given in table 1.

TABLE 1.—*Effect of tannic acid on the infectivity of buffered tobacco-mosaic virus at a dilution of* 1×10^{-2}

Concentration of tannic acid	Time virus exposed to acid	Virus diluted 1×10^{-2} in 0.1 M phosphate buffers				
		pH 10.0	pH 8.5	pH 7.0	pH 4.5	pH 3.0
Control		1617[a]	3468	3497	1605	1123
1.0 %	1 min.	475	512	245	28	30
"	5 "	395	172	97	9	8
"	10 "	134	204	48	3	4
"	15 "	121	196	39	0	0
"	30 "	135	93	31	0	0
"	60 "	0	0	0	0	0
0.5 %	" "	294	250	138	3	4
0.1 %	" "	2234	3217	1656	37	68
0.05%	" "	2587	3593	3420	551	553
0.01%	" "	3053	4467	4547	1891	1567
Check		0	0	0	0	0

[a] = Average number of lesions on 20 beans.

The data show that activity in an alkaline medium is inhibited by a 1 per cent solution of tannic acid in 1 hour and in an acid medium in 15 minutes. They also show that the amount of inhibition of infection depends upon the concentration of tannic acid and the time of action. Reduction of activity was greater in acid than in alkaline suspensions.

EFFECT OF TANNIC ACID ON NONBUFFERED VIRUS

Nonbuffered virus at various dilutions was treated with tannic acid at different concentrations and then inoculated to 20 Scotia bean plants without adjusting the reaction or the concentration of tannic acid. Control plants were inoculated with virus suspensions at comparable dilutions in distilled water.

The results (Table 2) indicates that activity, regardless of virus concentration, was inhibited in 15 minutes at room temperature by tannic acid in 1 per cent concentration, while virus treated for 1 to 10 minutes was slightly infectious. In solutions of less concentration of acid (0.5, 0.1, 0.05, and 0.01 per cent) virus infectivity, after an exposure of 1 hour, was inversely proportional to the concentration of acid. Virus exposed to a .5 per cent solution of the acid for 5 to 20 days was noninfectious. It is not

TABLE 2.—*Effect of tannic acid on the infectivity of nonbuffered tobacco-mosaic virus at various dilutions*

Virus dilution	Reaction of suspension	Concentration of acid	Time of action										
			1 min.	5 min.	10 min.	15 min.	30 min.	60 min.	6 hrs.	1 day	5 days	10 days	20 days
0.5×10^{-1}	pH 5.4	1.0%	18[a]	6	3	0	0	0					
"	"	Control (none)						12,390					
1.0×10^{-1}	pH 5.8	1.0%	12	9	2	0	0	0					
"	"	Control (none)						4,955					
1.0×10^{-2}	pH 6.2	1.0 %	106	15	11	0	0	0					
"	"	0.5 %	487	283	214	93	28	10	78	4	0	0	0
"	"	0.1 %	864	555	341	226	107	46	20	24	18	16	30
"	"	0.05%	1437	1269	1108	1235	1093	986	492	181	133	22	46
"	"	0.01%	2329	1896	1941	1637	1728	1894	748	855	453	780	384
"	"	Control (none)						1872	826	985	769	890	472
Check			0	0	0	0	0	0	0	0	0	0	0

[a] = Average number of lesions on 20 beans.

evident from the data whether virus was inactivated appreciably by tannic acid at concentrations of 0.1, 0.05, and 0.01 per cent, since, on standing, the infectivity of the control sample was reduced to about the same degree as the acid samples. The reactions of the suspensions were measured colorimetrically.

RESTORATION OF VIRUS ACTIVITY UPON REMOVAL OF TANNIC ACID

(a) *By Ultrafiltration.* Activity of tannic acid-virus suspensions was partially restored upon removing the acid by ultrafiltration. Ten cc. of a noninfectious tannic acid-virus mixture were passed through collodion membranes (28.96 mμ pore diameter) which retained the virus but not the acid. After passing 20 cc. of distilled water through the material on the membrane to remove the remaining tannic acid, the residues were taken up in 10 cc. of 0.1 molar phosphate buffers at a reaction of pH 8.5 and inoculated to beans.

TABLE 3.—*Infectivity of virus in the residues after removal of tannic acid from noninfectious virus-tannic acid suspensions by ultrafiltration*

Virus dilution	Reaction of suspension	Concentration of tannic acid	Activity of virus				
			Unfiltered suspension	Residue of virus treated with tannic acid			
				1 day	5 days	10 days	15 days
1×10^{-1}	pH 5.8	None (control)	36,500[a]	26,500	22,500	21,000	19,530
"	"	1. %	0	24,000	24,000	18,000	17,650
"	"	2. %	0	21,500	26,500	20,500	21,440
"	"	5. %	0	16,500	21,500	16,500	16,300
"	"	10. %	0	19,750	23,500	19,500	19,250
1×10^{-2}	pH 6.2	None (control)	3,386	2,500	.....	.....	.....
"	"	1. %	0	1,827	.....	.....	.....
"	pH 8.5	None (control)	3,542	2,115	.....	.....	.....
"	"	1. %	0	1,370	.....	.....	.....

[a] = Average number of lesions on 20 Scotia beans inoculated at pH 8.5.

Ninety, 81, 62, and 74 per cent, respectively, (Table 3) of the activity was restored upon removing the acid from virus diluted 1×10^{-1} after treatment with 1, 2, 5, and 10 per cent tannic acid for 1 day, and 73 and 64 per cent of the activity was restored from virus diluted 1×10^{-2} at reactions of pH 6.2 and pH 8.5 after standing for 1 day in a 1 per cent solution of tannic acid. The virus was not permanently inactivated after standing 15 days in 10 per cent tannic acid.

(b) *By Precipitation with Gelatin.* Activity of tannic acid-virus suspensions was partially restored upon removing the acid by precipitation with gelatin. Two cc. of a 5 per cent solution of gelatin was added to 10

cc. of nonbuffered virus diluted 1×10^{-2} in 1 per cent solution of tannic acid. The materials were thoroughly mixed and inoculated to beans after periods of from 1 to 60 minutes. Similarly, noninfectious virus was treated with gelatin in 0.5, 0.1, 0.05, and 0.01 per cent concentrations and allowed to stand 1 hour before inoculating to beans. Control plants were inoculated with virus diluted 1×10^{-2} in distilled water, in a 1 per cent solution of tannic acid, and in a 1 per cent solution of gelatin.

The number of lesions produced from the inoculations indicated that 15 per cent of the original virus activity was restored within 1 minute by gelatin at 1 per cent concentration. From 24 to 64 per cent of the activity was recovered in 5 to 60 minutes in 1 per cent gelatin, while 41, 5, 1, and 1 per cent, respectively, was regained in 1 hour by 0.5, 0.1, 0.05, and 0.01 per cent gelatin. Virus diluted 1×10^{-2} in 1 per cent solution of gelatin produced 1834 lesions and that in water 1995 lesions on 40 bean leaves. Virus in a 1 per cent solution of tannic acid was noninfectious. It is apparent that a noninfectious tannic acid-virus suspension is made active upon precipitation of the acid with gelatin, and that the recovered activity appears to be proportional to the concentration of gelatin.

Effect of Tannic Acid on Infection by Virus Applied Either Before or After the Application of Acid

Inhibiting effect of tannic acid on infection when virus and the acid are applied separately to plants was determined in the following manner. The upper surfaces of the primary leaves of 20 bean plants were sprayed with 10, 5, 2, 1, 0.5, 0.1, 0.05, and 0.01 per cent solutions of tannic acid previous to inoculation with virus diluted 1×10^{-1} and 1×10^{-2} in distilled water. Other plants were inoculated with comparable samples of virus and the inoculated surfaces sprayed within 1 minute with 10, 5, 2, 1, 0.5, 0.1, 0.05, and 0.01 per cent solutions of the acid. Control plants not treated with the acid were inoculated with comparable samples of virus.

The results (Table 4) show that tannic acid, when applied in 0.01 to 10 per cent concentrations to the host plant before inoculations, greatly reduced infection, but in no case completely inhibited it, and that the degree of inhibition was proportional to the concentration of the acid. When applied in the same concentrations to plants after inoculation, it inhibited infection to a less degree. Plants inoculated with virus diluted 1×10^{-1} developed about 20 to 50 per cent as many lesions as the controls. Plants inoculated with virus diluted 1×10^{-2} developed about the same number of lesions as the controls, except those treated with acid at 1 per cent or greater concentrations. Acid at these concentrations reduced the number of lesions about 25 to 50 per cent.

TABLE 4.—*Effect of tannic acid on infection by virus applied before or after the application of acid*

Dilution of virus	Concentration of acid	Plants sprayed with tannic acid		Check
		Before inoculation	After inoculation	
1×10^{-1}	No acid (control)	8100[a]	8100	0
	.01%	4450	4680	0
	.05%	865	3650	0
	0.1 %	76	4600	0
	0.5 %	23	4450	0
	1. %	15	3800	0
	2. %	11	2100	0
	5. %	4	1900	0
	10. %	3	1750	0
1×10^{-2}	No acid (control)	1639	1639	0
	.01%	1690	1735	0
	.05%	460	1920	0
	0.1 %	34	1286	0
	0.5 %	28	1320	0
	1. %	16	1185	0
	2. %	6	1092	0
	5. %	5	873	0
	10. %	6	941	0

[a] = Average number of lesions on 20 Scotia beans.

DISCUSSION

Inhibition of tobacco-mosaic virus infection by tannic acid appears to result, in part, from an interaction of virus and acid, since complete inhibition depends upon concentration of acid and time of action. However, a part of the effect may result from the action of tannic acid on the host plant. The mechanism of the action is not apparent, but some of the well-known properties of tannic acid (precipitation of soluble proteins, combination with proteins, and dehydration of emulsoid materials) suggest an explanation of the reaction. According to Kruyt,[6] dehydration of agar hydrosols by tannic acid results from the adsorption of hydrophobic tannic acid molecules on the surface of the hydrophilic agar particles and not from a chemical affinity of the acid for water. If the acid molecules were adsorbed on the surface of the infectious particles as on agar, the physical state of the virus might be altered sufficiently to prevent infection without any permanent change in the virus. Such a condition might be readily reversible upon removal of the acid. If the virus were protein in nature or its activity were associated with pro-

[6] Kruyt, H. R. Colloids. Trans. by H. S. van Klooster. Ed. 2. 286 pp. John Wiley and Sons, Inc., New York. 1930.

teins, combination of tannic acid and protein might be expected to change the infectious substances into a noninfectious one.

Since the measurement of virus depends upon infection, a phenomenon not clearly understood, it is difficult to conclude whether the inhibition of infection results from a change in the virus, in the protoplasm of the host plant, or both. The fact that infection occurs in the presence of 1 to 10 per cent tannic acid when inoculation is carried out within 10 minutes appears to be evidence that a part of the inhibition of infection results from a physical or chemical reaction between the virus and tannic acid. The reaction appears to be reversible, since the amount of regained virus activity after precipitation of the acid with gelatin depends upon the concentration of gelatin and time of action. Activity was also restored upon separating the acid from virus by ultrafiltration, but, since it required about 2 hours to complete this procedure, the rate of reactivation was not studied. Virus after standing for 15 days in 10 per cent tannic acid was capable of recovering its infectivity.

Inhibition of tobacco-mosaic virus infection by tannic acid differs from that by trypsin,[7] but is similar, in general, to the action of tannic acid on the virus of equine encephalomyelitis.[8]

SUMMARY

1. Inhibition of tobacco-mosaic virus infection by tannic acid depends upon the concentration of acid and time of action.

2. Activity is restored upon removal of tannic acid from the virus suspensions by precipitation with gelatin or by ultrafiltration. Restored activity was proportional to the amount of gelatin added and time of action. Virus, after standing for 15 days in 10 per cent tannic acid, was made active by filtering off the acid.

3. Inhibition of infection on plants treated with tannic acid before inoculation was proportional to the concentration of acid. Tannic acid at concentrations of 0.01 to 10 per cent had but slight effect upon infection when applied to the plants after inoculation.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

[7] See footnote 2.

[8] See footnote 4.

PARTICLE DIAMETER OF CERTAIN PLANT VIRUSES AND PHYTOMONAS PRUNI BACTERIOPHAGE[1]

H. H. THORNBERRY

(Received for publication June 22, 1935)

INTRODUCTION AND HISTORICAL REVIEW

Determination of particle diameters of viruses by ultrafiltration methods has been greatly aided the last few years by Elford's (6) type of collodion membranes and its modification by Bauer and Hughes (2). Following Elford's (6) method, MacClement and Smith (17) estimated the viruses of Johnson's tobacco mosaics 1 and 6, aucuba mosaic, and Hyoscyamus mosaic to have particle diameters of 15, 15, 40–50, and 150 mμ[2], respectively. Smith (19), using the same method, reported potato x and y viruses to have diameters 200 mμ and 250 mμ, respectively. He (19) also mentioned that tomato spotted wilt and tobacco ring spot viruses passed through membranes of 350 mμ pore diameter, but were retained by those of 194 and 220 mμ, respectively. Duggar and Karrer (5) by ultrafiltration methods with alcohol-ether collodion membranes reported tobacco-mosaic virus to have a diameter of 30 mμ. By ultracentrifugal analysis Bechhold and Schlesinger (3) found that tobacco-mosaic virus had a diameter of 50 mμ, and Waugh and Vinson (22), by coefficient-of-diffusion analysis, reported its diameter to be 10 mμ. The purpose of this paper is to report results obtained from experiments designed to determine particle diameters of certain plant viruses and of *Phytomonas pruni* (E. F. Smith) Bergy *et al.* bacteriophage.[3]

METHODS

Viruses from the following diseases were used:

Johnson's (14) tobacco mosaic 1 and 6.
Holmes' (11) masked tobacco mosaic.
Bewley's (4) aucuba mosaic of tomato.
Kunkel's (16) second attenuated strain of aucuba mosaic.
Jensen's (12) yellow tobacco mosaic 101, 102 and 7a.
Valleau's (21) yellow ring spot of tobacco.
" (21) green " " " "
Wingard's ring spot of tobacco described by Fromme, Wingard, and Priode (9).

[1] Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by date of receipt of the manuscript.

[2] mμ = millimicron, 1×10^{-9} meter.

[3] *P. pruni* bacteriophage was obtained from Dr. H. W. Anderson, University of Illinois, who kindly supplied unpublished data on the procedure for producing lysis of the bacteria.

Porter's (18) cucumber mosaic, the virus of which was designated as "cucumber virus 1."
Johnson's (13) ring spot of potato.
Stanley's[4] purified tobacco mosaic 2/19/35.

All the virus samples were obtained from plants of *Nicotiana tabacum* L. var. Turkish that had been infected 3 or 4 weeks with the given virus. Anderson's (1) *Phytomonas pruni* bacteriophage was obtained from broth cultures. Infected plants were macerated with a food chopper and the infectious juice frozen in test tubes until needed for the experiments. Because of differences in infectivity of the original juices the samples were diluted to different degrees in order to equalize, as far as possible, the infectivity of the suspensions used for ultrafiltration. A given amount of the infectious juice (number of ml. to give the desired dilution in 100 ml. of liquid), after being frozen and thawed on three occasions, was added to 20 ml. of nutrient broth. This mixture was diluted to 100 ml. and adjusted to 0.1 molar phosphate salt concentration and a reaction of pH 8.5. At this reaction a flocculent precipitate formed and settled upon standing. The supernatant liquid after complete sedimentation of the precipitate was carefully decanted and filtered through Seitz E. K. filter pads under 700 mm. of mercury-negative pressure. Ten ml. of the Seitz filtrate were then passed through the collodion membranes of various pore diameters under 1 atmosphere of positive pressure obtained by compressed nitrogen gas. Previous to ultrafiltration, 2 ml. of broth that had been filtered through a membrane of 20 mμ pore diameter were passed through each membrane to saturate the areas of high surface energy.

Virus activity of the various samples was determined by inoculation to appropriate test plants. That of concentrated samples was determined by inoculating aliquots diluted to the extent of giving less than 300 lesions per leaf and the actual number of lesions produced multiplied by the dilution value. The plants used for each virus are mentioned in a footnote to table 2. Before inoculating Turkish tobacco plants, the reaction of the samples was adjusted from pH 8.5 to 6.5. Other plants were inoculated at pH 8.5. A small amount of carborundum powder No. 320 was added to the ring-spot and cucumber-mosaic samples before inoculation to cowpeas. Turkish tobacco plants, inoculated with samples of Holmes' masked tobacco mosaic were macerated and the juice rubbed on Scotia bean leaves to determine the presence of virus in the symptomless plants. All the plants were washed with running tap water after inoculation.

The samples of bacteriophage used were obtained from 16-hour-old

[4] Most samples were taken directly from mosaic-diseased plants; but one sample, purified by the lead acetate procedure, was obtained from Dr. W. M. Stanley.

broth cultures of *Phytomonas pruni* that had been lysed by the bacteriophage for 18 hours at 35° C. and a reaction of pH 7.2–7.4. This suspension was adjusted to pH 8.5 in 0.1 molar phosphate buffer and passed through Seitz E. K. filter pads under 700 mm. of mercury-negative pressure. Ten ml. of the Seitz filtrate free of bacteria and active at a dilution of 1×10^{-5} were passed through a graded series of collodion membranes to determine the end point of ultrafiltration. Activity of the ultrafiltrates was determined by adding 5 ml. of each sample after being adjusted to pH 7.2–7.4 to 5 ml. of a 16-hour-old broth culture of the organism. Lysis, as evidenced by clearing of the suspension after 18 hours, indicated the presence of bacteriophage and was designated by ++ signs in table 2. Utrafiltrates that

TABLE 1.—*Membrane data for the calculations of pore radius*

Membrane number	Per cent acetic acid	Thickness of membrane cm.	Coefficient of viscosity of water at 22° C., C.G.S.	Volume of water passing, ml./sec.	Pressure of water during flow dynes/sq. cm.	Volume of pores, ml.	Radius of pores, mμ.
63	0.40	0.018	0.00961	0.03561	399,600	0.1029	46.44
61	0.80	0.018	"	0.019005	"	0.1015	34.16
87	1.00	0.022	"	0.006205	"	0.1141	22.50
85	1.10	0.020	"	0.006594	"	0.1337	19.48
73	1.15	0.018	"	0.004663	"	0.1015	16.92
81	1.20	0.017	"	0.003003	"	0.0931	13.39

failed to produce visible lysis, were passed through Seitz E. K. filter pads and added to 5 ml. of a fresh broth culture of the organism. Visible clearing of these cultures, designated by a + sign, indicated that the ultrafiltrates contained bacteriophage in too small a concentration for lysis. Ultrafiltrates that failed to produce lysis in the second culture were considered to be free of active bacteriophage particles and were designated by a 0 sign.

Preparation of Membranes

The membranes were prepared according to the formula given by Bauer and Hughes (2) since Mallinckrodt's parlodion was used as the source of collodion instead of "Nicol" used by Elford (6). Constituents of the stock solution were:

Parlodion	150 grams
Absolute ethyl alcohol	250 "
Anhydrous ethyl ether	750 "
Acetone	1150 "
Iso-amyl alcohol	575 ml.

TABLE 2.—*Ultrafiltration of a certain plant viruses and Phytomonas pruni through collodion membranes of graded pore diameters*

Infectious agents	Sample		Unfiltered suspension	Seitz filtrate	Ultrafiltrates through membranes, pore diameters in millimicrons						Check
	pH	Dilution			92.88	68.32	45.00	38.96	33.84	26.27	
Johnson's tobacco mosaic #1	8.5	$1. \times 10^{-1}$	101,200[a]	131,300	3,100	585	141	0	0	0	0
			5[b]	5		5	5	0	0	0	0
Johnson's tobacco mosaic #6	8.5	$1. \times 10^{-1}$	96,500[a]	143,000	1,310	425	85	0	0	0	0
			5[b]	5		5	4	0	0	0	0
Holmes masked tobacco mosaic	8.5	1.66×10^{-1}	85,400[a]	128,500	850	540	118	0	0	0	0
			5[c]	5		5	4	0	0	0	0
Smith's aucuba mosaic of tomato	8.5	1.25×10^{-1}	87,900[a]	126,000	970	320	119	0	0	0	0
			5[b]	5	5	5	5	0	0	0	0
Kunkel's aucuba mosaic	8.5	1.25×10^{-1}	96,000[d]	124,000	1,500	235	71	0	0	0	0
			93,300[a]	132,600	1,600	640	160	0	0	0	0
			5[b]	5	5	5	5	0	0	0	0
Jensen's yellow tobacco mosaic #101	8.5	2.5×10^{-1}	102,500[d]	145,000	970	300	24	0	0	0	0
			82,700[a]	125,000	240	123	12	0	0	0	0
			5[b]	5	5	5	4	0	0	0	0
Jensen's yellow tobacco mosaic #102	8.5	2.5×10^{-1}	79,800[a]	112,000	450	125	58	0	0	0	0
			5[b]	5	5	5	5	0	0	0	0
Jensen's yellow tobacco mosaic #7a	8.5	5.0×10^{-1}	9,700[a]	13,000	157	13	4	0	0	0	0
			5[b]	5	5	5	2	0	0	0	0
Valleau's yellow ring spot of tobacco	8.5	3.3×10^{-1}	5[b]	5	5	5	5	0	0	0	0
			24[e]	190	155	131	23	0	0	0	0

TABLE 2.—(*Continued*)

Infectious agents	Sample		Unfiltered suspension	Seitz filtrate	Ultrafiltrates through membranes, pore diameters in millimicrons						Check
	pH	Dilution			92.88	68.32	45.00	38.96	33.84	26.27	
Valleau's green ring spot of tobacco	8.5	3.3×10^{-1}	5[b]	5	5	5	5	0	0	0	0
Wingard's ring spot of tobacco	8.5	3.3×10^{-1}	1,206[c]	840	740	350	48	0	0	0	0
Porter's cucumber mosaic #1	8.5	3.3×10^{-1}	5[b]	5	5	5	4	0	0	0	0
Johnson's ring spot of potato	8.5	2.5×10^{-1}	565[c]	870	190	122	35	0	0	0	0
Stanley's purified tob. mos. virus 2/19/35	8.5	$5. \times 10^{-1}$	5[b]	5	5	5	5	0	0	0	0
Anderson's *P. pruni* bacteriophage	8.5	$5. \times 10^{-1}$	12[c]	9	7	6	2	0	0	0	0
			5[b]	5	5	5	5	0	0	0	0
			12,560,000[a]	11,845,000			853	459	28	0	0
			++[f]	++	++	++	+	+	+	0	0

[a] Average number of lesions on 40 Scotia bean leaves.
[b] Number of diseased plants from 5 Turkish tobacco plants inoculated.
[c] Number of plants containing virus from 5 Turkish tobacco plants inoculated.
[d] Average number of lesions on 15 *Nicotiana sylvestris* leaves.
[e] Average number of lesions on 40 Black Eye cowpea leaves.
[f] Lysis of both cultures of *Phytomonas pruni* by bacteriophage.

The stock solution of collodion, after the addition of an equal volume of a mixture of 1 part of alcohol and 9 parts of ether by weight, was divided into 200 ml. portions and the amount of glacial acetic acid, indicated in table 1, was added to regulate the pore size. After thoroughly mixing, each solution was poured into leveled (by spirit levels) glass cells, 40 cm. in diameter, similar to those used by Bauer and Hughes (2). After allowing the solution to evaporate 75 minutes at 22° C. in a room with still air containing 60 per cent relative humidity, the glass cells with partially solidified collodion were submerged in distilled water until the collodion loosened (about 15 minutes) from the glass. After washing the membranes for 14 days with 3 changes of distilled water per day, small discs 3.5 cm. in diameter were cut from the central portion of the collodion sheet, and stored in distilled water until needed for ultrafiltration.

EXPERIMENTATION

Calibration of Membrane Pore Diameter

Pore diameters of the various membranes prepared were calculated according to the formula given by Elford (6):

$$r - 2l\sqrt{\frac{2\,n\,q}{p\,v}}, \text{ where:}$$

r—radius of pores in cm.
l—length of capillary, thickness of membrane in cm.
n—coefficient of viscosity of water at temperature of measurement in C. G. S. units.
q—volume of water passing in ml./sec.
p—pressure producing flow in dynes/sq. cm.
v—total volume of pores (assumed equivalent to water content of membrane).

Data (Table 1) necessary for the calculation of pore radius by this formula are given for membranes near the end point of ultrafiltration. Pore diameter instead of radius is used hereafter in this paper.

End Point of Ultrafiltration and Estimation of Particle Diameter

The end point of ultrafiltration of each virus and the bacteriophage, the smallest pores that allow the infectious particles to pass, was determined by filtering the samples under controlled conditions through a graded series of membranes. Infectivity of the ultrafiltrates indicates the membranes that allowed the infectious particles to pass. Data for membranes near the end point of ultrafiltration are given in table 2.

The data show that all the viruses in these experiments passed membranes of 45 mμ pore diameter, but were retained by membranes of 38.96 mμ pore diameter when the samples were not purified. According to Elford (7) who mentioned that particles were not greater than 0.33 the size of the

pores through which they passed, each of the viruses is estimated to have a diameter of 15 mμ. Tobacco-mosaic virus in samples purified by the lead-acetate procedure and concentrated to the extent of being active at a dilution of 1×10^{-6} and *Phytomonas pruni* bacteriophage passed through membranes of 33.84 mμ pore diameter, but were retained by membranes of 26.27 mμ pore diameter. The diameters of these infectious agents under these conditions are estimated to be 11 mμ.

DISCUSSION

Ultrafiltration analysis according to these results affords no aid in the classification of plant viruses on the basis of particle size, since all the unpurified suspensions contained infectious particles of approximately the same diameters. Particle diameters of some animal and plant viruses as well as bacteriophages, however, have been reported by several investigators to differ in size when estimated by the ultrafiltration analysis. Whether the factors that affect the passage of virus through the membrane interstices were sufficiently controlled by other investigators to estimate the minimal diameter of each virus, or whether the factors were adequately controlled in these studies to show diameter differences is not apparent.

An attempt was made in these studies to keep the conditions during ultrafiltration and the procedure in preparing the samples uniform so as to minimize, as far as possible, differences that might affect the passage of virus through the membranes. Adsorption of virus to the negatively charged membranes was eliminated, or greatly reduced, by adjusting the suspension to a reaction of pH 8.5 at which the viruses probably are negatively charged. Streaming potential between the membrane and suspension during the flow of liquid was prevented by the presence of ionizable phosphate buffer salts. Areas of high surface energy in the membranes capable of holding virus were saturated with the surface-tension-reducing substances in broth previous to ultrafiltration and an attempt was made to counteract differences in capillary-active substance, if any, in the various virus juices by adding 20 per cent broth to the suspensions. Gelatin, sodium oleate, and saponin, substances that also possess surface-tension-reducing properties and facilitate ultrafiltration, were inferior to broth in aiding ultrafiltration.

Physical properties of the viruses prepared by different procedures might have influenced the ability of the particles to pass through membrane interstices of irregular shape and thus account for the discrepancy between the particle diameter values found by MacClement and Smith (17) and those obtained in these studies for aucuba mosaic. It is well known that differently prepared samples of proteins may possess distinct physical properties. Reaction of the medium according to Svedberg's (20) sedi-

mentation analysis of proteins affects the molecular weight or particle size of proteins that have a characteristic stable zone. When the acid border of the stability region is exceeded denaturation and aggregation take place, and when the alkaline border is exceeded disintegration occurs. Within the stable zone a change of reaction does not affect the particles, as is shown by sedimentation analysis. Results from previous filtration and infectivity studies on tobacco mosaic suggest that the virus in 0.1 molar phosphate buffer at a reaction of pH 8.5 is dispersed to a greater degree than in non-treated juice. Conditions that affect dispersion may influence the estimation of particle diameter by ultrafiltration methods. Whether the reaction and concentration of salt of the suspensions affect particle hydration and in this way influence the estimation of particle diameter is not apparent. Hydration influences the values of molecular diameter of substances measured by certain methods, according to Kunitz, Anson, and Northrop (15). Elasticity of oil-emulsion particles, according to Hatschek (10), determines their passage through membrane pores smaller than the particles when sufficient pressure is applied. Whether the virus particles possess sufficient elasticity to change in shape under pressures applied during ultrafiltration is not apparent. Concentration of virus affects the end point of ultrafiltration, according to Elford and Andrews (8), and may account for the discrepancy in results. Tobacco-mosaic virus purified by the lead-acetate procedure and concentrated to the extent of being active at a dilution of 1×10^{-6} passed membranes of 33.84 mμ pore diameters, while nonpurified virus active at a dilution of 1×10^{-4} was retained by these membranes.

SUMMARY

1. Thirteen plant viruses and virus strains in nonpurified samples were estimated by ultrafiltration analysis to have diameters of about 15 mμ. One of these, tobacco-mosaic virus, in purified preparations contained particles estimated to be 11 mμ in diameter.

2. Particles of *Phytomonas pruni* bacteriophage were estimated to be 11 mμ in diameter.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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CLASSIFICATION OF SOUTHERN CELERY-MOSAIC VIRUS[1]

W. C. PRICE

(Accepted for publication July 24, 1935)

A mosaic disease of celery was found in Florida in 1924 by Foster and Webber (3). It has since been studied extensively by Doolittle (1), by Doolittle and Wellman (2), and by Wellman (6, 7, 8), and is commonly designated as southern celery mosaic. In a recent paper Wellman (6) pointed out the similarities and differences between southern celery mosaic and certain other plant-virus diseases. His results led him to conclude that the virus of this disease is specifically different from any other heretofore described, and he classified it as celery virus 1. Although similar to the common cucumber-mosaic virus in many respects, it was considered to be distinct from this virus because of certain striking differences in symptomatology and host range, and because of its slightly higher thermal death point and greater ability to withstand aging *in vitro*.

Recently, the writer (5) has described a technique for the demonstration of a specific immune reaction to cucumber mosaic in *Zinnia elegans* Jacq. and has suggested the usefulness of the technique for plant-virus classification purposes. Because of the marked similarities between southern celery mosaic and cucumber mosaic, it seemed desirable to make a comparison of the two from an immunological standpoint. Experiments were undertaken for the purpose of determining whether or not zinnia plants infected with celery-mosaic virus would become specifically immune from infection with cucumber-mosaic virus. In addition, a comparison was made of symptoms produced by the two viruses in certain host plants.

IMMUNOLOGICAL RELATIONSHIPS

A yellow strain of cucumber-mosaic virus, referred to here and in previous publications (4, 5) as strain 6, produces such conspicuous necrotic primary lesions in *Zinnia elegans* var. Golden Gem Midget that inoculation with it affords a convenient test of immunity in this plant. It was recently shown (5) that plants mottled by any one of several different strains of cucumber-mosaic virus are immune from virus of strain 6, but that plants infected with unrelated viruses, such as tobacco mosaic, are not immune from cucumber-mosaic-strain-6 virus. It was likewise shown that zinnia plants infected with tobacco- or aucuba-mosaic viruses become immune from a necrotic-type tobacco-mosaic virus (strain 302A), but that similar plants infected with cucumber-mosaic or other viruses unrelated to that of tobacco mosaic are not immune from this necrotic-type virus.

[1] Published at the expense of The Rockefeller Institute for Medical Research out of the order determined by date of receipt of the manuscript.

The plan of the present experiments was to inoculate zinnia plants with virus of southern celery mosaic,[2] to allow them to become thoroughly mottled, and finally, after a period of 20 to 30 days, to inoculate them with virus of cucumber mosaic strain 6. A number of such experiments were conducted, all inoculations being made by means of the rubbing method. Table 1 presents the results of two experiments, which show that virus of southern celery mosaic completely protects zinnia plants against cucumber mosaic strain 6.

TABLE 1.—*Numbers of lesions produced in diseased and healthy plants of zinnia by inoculation with cucumber mosaic strain 6 and tobacco mosaic strain 302A*

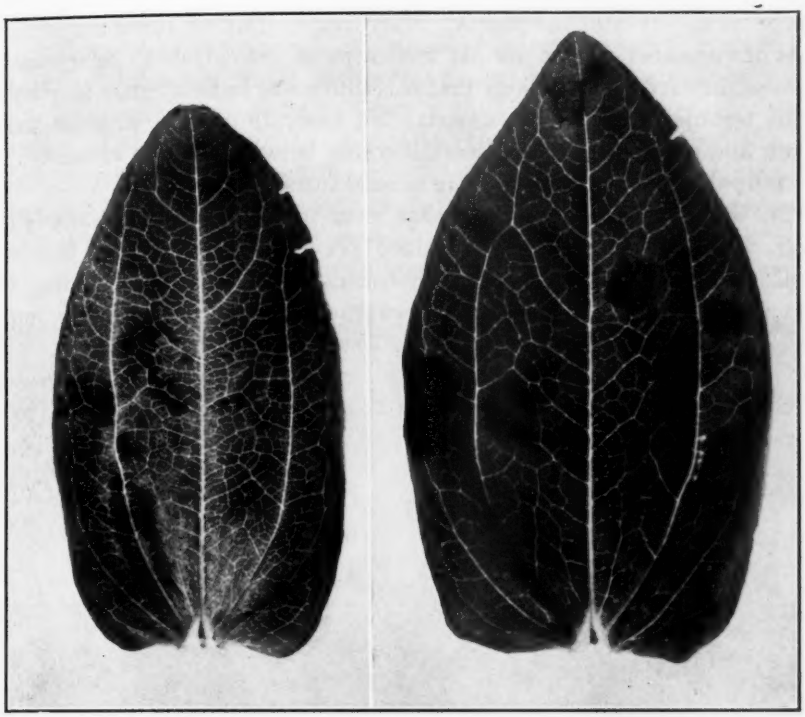
Plant No.	Lesions produced by inoculation with cucumber mosaic strain 6				Lesions produced by inoculation with tobacco mosaic strain 302A			
	Test 1		Test 2		Test 1		Test 2	
	Test plants	Controls	Test plants	Controls	Test plants	Controls	Test plants	Controls
1	0	86	0	5	7	39	23	46
2	0	51	0	1	0	51	38	42
3	0	26	0	2	0	16	14	63
4	0	50	0	5	7	16	42	30
5	0	46	0	3	4	20	61	27
6	0	25	0	2	7	36	45	57
7	0	23	0	2	11	10	12	36
8	0	36	0	3	3	5	23	56
9	0	65	0	2	4	57	5	63
10	0	51	0	14	29	17	17	28
Total	0	459	0	39	72	267	280	448

Figure 1 shows two zinnia leaves 7 days after they were inoculated with virus of cucumber mosaic strain 6. The leaf on the left was previously infected with celery virus 1, while that on the right was a healthy control. Necrotic lesions have developed only in the control leaf.

In another set of experiments, young zinnia plants were inoculated with celery-mosaic virus, allowed to become thoroughly mottled, and, finally, were inoculated with virus of tobacco mosaic strain 302A. The results of two experiments are given in table 1. They show that zinnia leaves mottled by celery-mosaic virus are not immue from infection with virus of tobacco mosaic strain 302A. It will, however, be seen from the table that fewer lesions appeared in the celery-mosaic-diseased plants than in the controls. This reduction in numbers of lesions is not specific but occurs when zinnia plants are infected with any one of a number of different viruses (5).

The results of the foregoing experiments show that zinnia plants infected with southern celery-mosaic virus become specifically immune from infec-

[2] A sample of southern celery-mosaic virus was kindly supplied by Dr. F. L. Wellman.



Photographed by J. A. Carlile.

FIG. 1. Leaves of *Zinnia elegans* Jacq. after inoculation with virus of cucumber mosaic strain 6. The leaf on the left was previously infected with southern celery-mosaic virus and shows the chlorois produced by this virus. It did not develop lesions when inoculated with virus of cucumber mosaic strain 6. The leaf on the right was a healthy control and has developed a number of necrotic lesions.

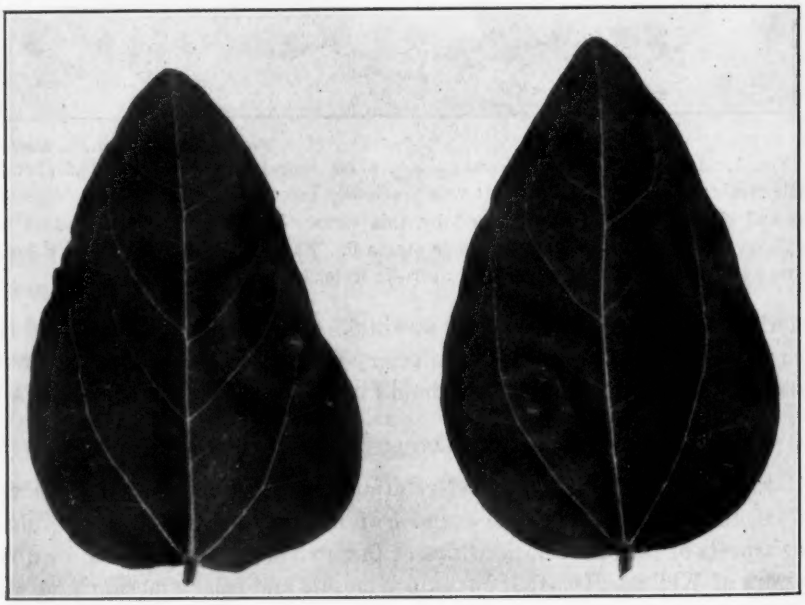
tion with virus of cucumber mosaic strain 6. It, therefore, is concluded that, from an immunological standpoint, celery-mosaic virus is closely related to cucumber-mosaic virus and that it should be classified as a strain of the latter.

HOST AND SYMPTOMATOLOGICAL RELATIONSHIPS

Because of the immunological relationship between celery-mosaic virus and cucumber-mosaic virus, it seemed of interest to examine more closely some aspects of the host relationships of these viruses. It is well known from the work of Wellman (6) that cucumber mosaic and celery mosaic have many characteristics in common. On the other hand, one of the criteria used by Wellman (6, 7, 8) for differentiating between celery mosaic and cucumber mosaic was the apparent difference in host range. While there has been no report of transmission of cucumber mosaic to hosts outside the Dicotyledoneae, it has been shown (8) that celery mosaic may infect at least 4 fam-

ilies of monocotyledonous plants. Moreover, certain of the dicotyledonous hosts of cucumber mosaic are not known to be susceptible to celery mosaic. It seems, however, not unlikely that this difference in host range may be due to the technique involved in determining susceptibility, or to some similar factor, and not primarily to a real difference between the two viruses. This has, indeed, proved to be the case in several instances.

The symptoms of cucumber-mosaic virus in cowpea, *Vigna sinensis* (L.) Endl. var. Black, have been described previously (4). These consist of small, dark brown, necrotic, primary lesions in the inoculated leaves. The lesions appear within 2 days after inoculation and are not, as a rule, numerous. Although cowpea was listed by Wellman (8) as giving a negative response to inoculation with celery-mosaic virus, the writer has found the virus to be readily transmitted to the Black variety of cowpea by the rubbing method of inoculation. The symptoms produced consist of primary lesions that are slightly smaller than, but in other respects identical with, those caused by cucumber-mosaic virus. Cowpea leaves bearing lesions produced by cucumber- and celery-mosaic viruses are illustrated in figure 2.



Photographed by J. A. Carlile.

FIG. 2. Leaves of cowpea, *Vigna sinensis* (L.) Endl. var. Black, showing necrotic primary lesions produced by southern celery-mosaic virus (left) and cucumber-mosaic virus (right).

Wellman (8) reported transmission of celery mosaic to creeping dayflower, *Commelina communis* L., by means of *Aphis gossypii* Glover, but was unable to obtain transmission to this host by leaf-rubbing methods of inoculation. The writer, using the rubbing method of inoculation, has obtained infection of *C. communis* not only with celery-mosaic virus but also with ordinary cucumber-mosaic virus and with virus of cucumber mosaic strain 6. The symptoms produced are illustrated in figure 3. Porter's cucumber-mosaic virus causes a pale, greenish yellow, blotch-like mottling. The mottling induced by celery-mosaic virus is more prominent and of a more

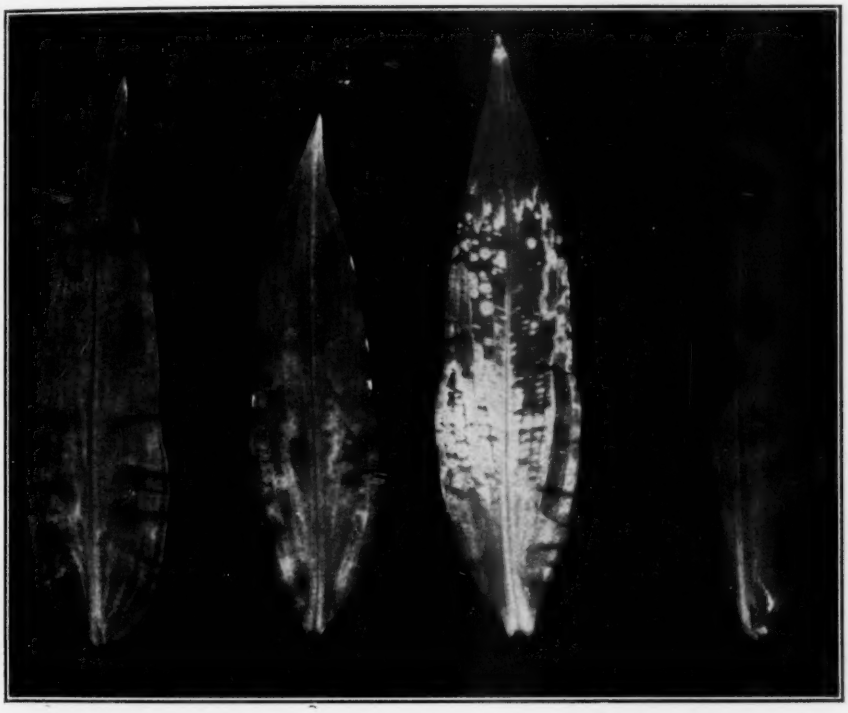


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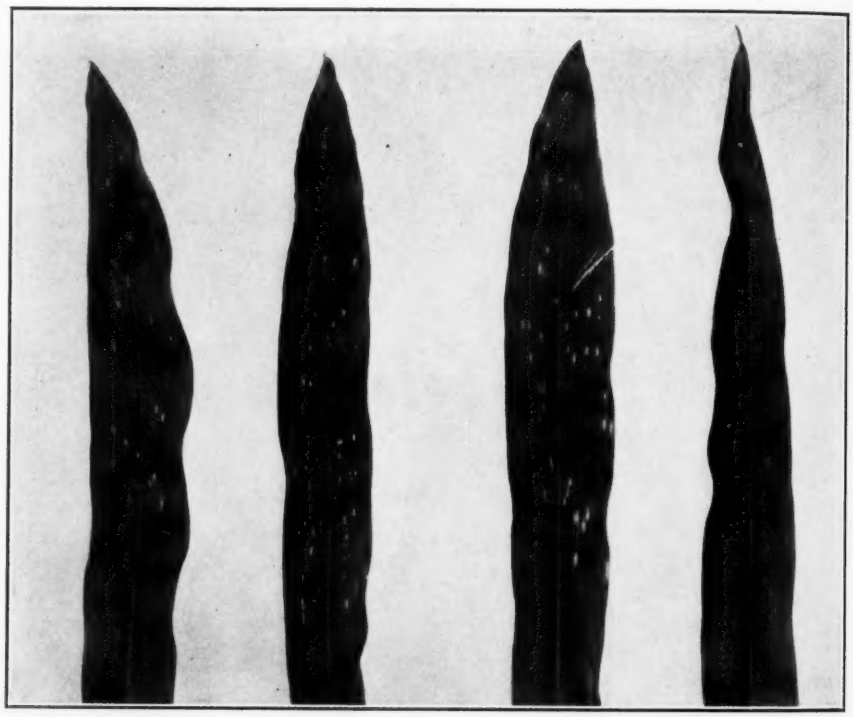
FIG. 3. Leaves of *Commelina communis* L. (reading from left to right): from a plant infected with southern celery-mosaic virus, a plant infected with cucumber-mosaic virus, a plant infected with cucumber-mosaic-strain-6 virus, and from a healthy plant.

pronounced yellow. The mottling elicited by cucumber-mosaic virus strain 6 is still more brilliant and is associated with or preceded by the production of bright yellow rings. The symptoms produced by the 3 viruses differ principally in the intensity of the mottling pattern. Each of the viruses was recovered from systemically diseased leaves of *C. communis* by subinoculations to tobacco.

By means of the rubbing method of inoculation, using carborundum as an abrasive, cucumber-mosaic, cucumber-mosaic-strain-6, and celery-mosaic viruses were transmitted to corn,[3] *Zea mays* L. Elliptical, chlorotic lesions appeared in the inoculated leaves, soon became necrotic, and spread downward producing tan, necrotic streaks (Fig. 4). Systemic symptoms did not develop in any of the plants tested. There appears to be no consistent difference between the 3 strains of virus as regards the type of primary lesions



Photographed by J. A. Carlile.

FIG. 4. Leaves of field corn, *Zea mays* L., after inoculation from tobacco with (reading from left to right): Southern celery-mosaic virus, cucumber-mosaic virus, cucumber-mosaic-strain-6 virus, and with juice from a healthy tobacco plant. Similar necrotic primary lesions appear on leaves inoculated with each of the 3 viruses.

they produce in this strain of corn. Each of the 3 viruses was successfully reisolated by making subinoculations to tobacco from necrotic primary lesions 9 days after the original inoculation of corn.

The symptoms produced by cucumber- and celery-mosaic viruses in cow-

[3] An inbred strain developed by J. R. Holbert and designated as R4.

pea, *Commelina communis*, and corn suggest a close relationship between the two viruses.

DISCUSSION

The fact that there is a specific immune reaction between celery-mosaic virus and cucumber-mosaic virus shows that they are closely related and suggests that they should be classified as strains of the same virus. The virus of celery mosaic appears to be more infectious than that of ordinary cucumber mosaic. Its greater infectiousness might explain its apparently wider host range and its seemingly higher thermal death point and greater ability to withstand aging *in vitro*. The symptomatological differences between ordinary cucumber mosaic and celery mosaic are no greater than those characteristic of other well-recognized strains of cucumber mosaic. In the present paper it has been shown that *Commelina communis* and *Zea mays*, both considered to be immune from cucumber mosaic, are, in fact, susceptible to this disease. Furthermore, it has been shown that the cowpea, which was previously regarded as immune from celery mosaic, is, in fact, susceptible. These findings suggest that the host range of cucumber mosaic and celery mosaic may eventually be found to be identical. The evidence presented in this paper furnishes a striking example of the usefulness of immune reactions for the purpose of arriving at an understanding of plant-virus relationships.

SUMMARY

Infection of zinnia plants with southern-celery-mosaic virus induces in them a specific immunity from a yellow strain of cucumber-mosaic virus (strain 6). Celery-mosaic virus and cucumber-mosaic virus, therefore, are closely related immunologically and, it is believed, should be classified as strains of the same virus. Corroborative evidence is found in the fact that the symptoms produced by celery-mosaic virus in *Zea mays* L., *Commelina communis* L., and *Vigna sinensis* (L.) Endl. are similar to those produced by ordinary cucumber-mosaic virus in the same hosts.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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PHYTOPATHOLOGICAL NOTES

Peronospora in Storage Cabbage.—In 1926, Thung[1] reported on infection of full-grown cabbage heads by *Peronospora parasitica* (Pers.) de Bary in Holland and stated that this type of infection had not been mentioned in the literature. He also noted that the fungus was able to spread in the heads and sometimes affect the parenchyma of the stems in storage cabbage. Since that time Weber[2] has reported that all of the foliage leaves and the exposed head leaves of Florida cabbage in the field frequently are affected with downy mildew; but, so far as the writer has been able to determine, no mention has been made of the storage type of infection in this country. It is the purpose of this note to describe and illustrate a serious kind of peronospora infection of storage cabbage in Wisconsin.

On inspection of cabbage that had been in storage for about 4 months in a Milwaukee warehouse it was discovered that from 15 or 40 per cent of the stock in some bins showed badly discolored leaves and stems (Fig. 1, A, B, F). In some instances the grayish black discolorations suggested black rot, but on more careful examination it was apparent that this trouble was in the parenchyma tissues and not associated with the vascular elements, as in the case of black rot (Fig. 1, F, G). Furthermore, microscopic examination of free-hand sections of affected tissues revealed the presence of large haustoria within the cells. The relatively inconspicuous intercellular hyphae and the large haustoria were essentially identical with the description and illustrations given by Gardner[3] for *Peronospora parasitica* in storage turnips.

From the specimens studied it was apparent that the fungus had worked its way from affected lower leaves into the stem, followed the parenchyma tissues into the pith region, and from there spread upward into the head. In some heads the fungus reached the terminal bud and the smallest innermost leaves were diseased (Fig. 1, C–E). Elsewhere, along the length of the stem, the fungus occasionally spread out from the pith through the parenchyma into the midribs of the head leaves and caused serious grayish black discoloration of the midrib and large portions of the leaf blades (Fig. 1, A, B). The mycelium from such invaded leaves often came to the surface and, by contact, caused infection of the neighboring leaves. In the moist air spaces between the head leaves the fungus often produced a scanty mycelium and conidiophores bearing spores typical for this organism.

[1] Thung, T. H. *Peronospora parasitica* (Pers.) De By. attacking cabbage heads. Phytopath. 16: 365–366. 1926.

[2] Weber, G. F. Some diseases of cabbage and other crucifers in Florida. Fla. Agr. Expt. Sta. Bul. 256, p. 21. 1932.

[3] Gardner, M. W. Peronspora in turnip roots. Phytopath. 10: 321–322. 1920.

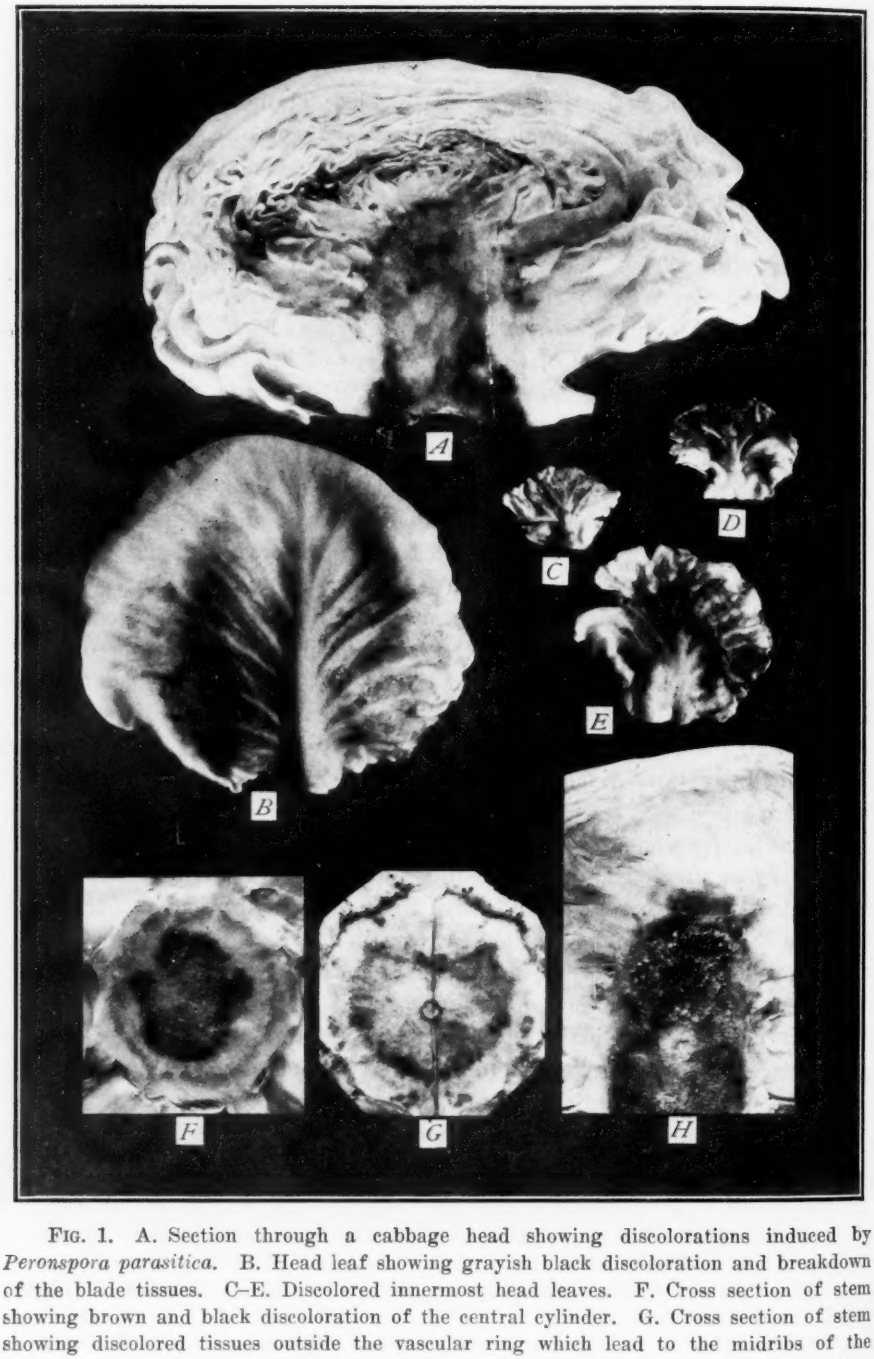

FIG. 1. A. Section through a cabbage head showing discolorations induced by *Peronspora parasitica*. B. Head leaf showing grayish black discoloration and breakdown of the blade tissues. C–E. Discolored innermost head leaves. F. Cross section of stem showing brown and black discoloration of the central cylinder. G. Cross section of stem showing discolored tissues outside the vascular ring which lead to the midribs of the outer head leaves. H. Longitudinal section of stem showing development of mycelium bearing conidiophores and conidia produced within 3 days in a humid atmosphere at 45°–50° F.

Small black spots, less than a millimeter in diameter, scattered over the surface of the leaves, suggested the possibility that they were the result of minor infections caused by germinating conidia. On the cut surfaces of affected heads placed in a refrigerator (45°–50° F.) typical conidiophores and conidia were produced within 3 days (Fig. 1, H). The conidia ranged from 22.5 to 30.0 μ in length and from 20.0 to 25.0 μ in width, the average size being 23.3×27.1 μ. No oospores were found in any of the affected heads of cabbage.

The seriousness of this disease as a storage and market trouble is readily apparent. In addition to the discoloration and decay of the tissues induced by Peronospora, the affected areas are very susceptible to secondary invasion by bacteria and such fungi as Alternaria and Rhizopus. In the storage lots under consideration great loss was occasioned by bacterial soft rot and alternaria rot following in the tissues affected by Peronospora. By excessive trimming some of the slightly affected heads were salvaged, but in many instances the salability of the stock was so affected that it was not worth the additional labor involved.

From the grower's standpoint it is significant that, in Holland, this fungus has been found to overwinter in old heads, leaves, and stumps of cabbage left in the field. Whether conditions in the cabbage-growing sections of Wisconsin permit the overwintering of the fungus in that manner is not known, but it is not unlikely that in some seasons it would be possible. The fact that this disease has not been reported in storage cabbage would indicate that either this type of infection is not common in northern-grown cabbage or it has been confused with other disease symptoms and not recognized as a separate disease. The potential seriousness of peronspora infection of full-grown cabbage warrants a thorough field and storage study of this fungus and the various types of disease symptoms induced.—G. B. RAMSEY, Bureau of Plant Industry, United States Department of Agriculture.

Toxicity of Low Concentrations of Ammonia to Mycelium and Sclerotia of Sclerotium rolfsii.—Since 1931 Southern sclerotium root rot caused by *Sclerotium rolfsii* Sacc. has been recognized as a serious disease of sugar beets in the Sacramento Valley of California. Measures for the prevention of distribution of the causal fungus and for the reduction of infestation by rotation are in operation, but satisfactory control of the disease is delayed by the absence of any effective method aimed directly at suppression of the fungus or prevention of infection.

Aqueous solutions of ammonia in concentrations as low as 50 ppm. of NH_3 for exposures of 24 hours have been found lethal to the mycelium of *S. rolfsii* in laboratory tests. Increasing the concentration to 150 ppm. of

NH_3 reduced the necessary period of exposure to 2 hours. The relation of concentration to the minimum lethal period is shown in accompanying figure 1. The minimum concentrations necessary to kill the more resistant

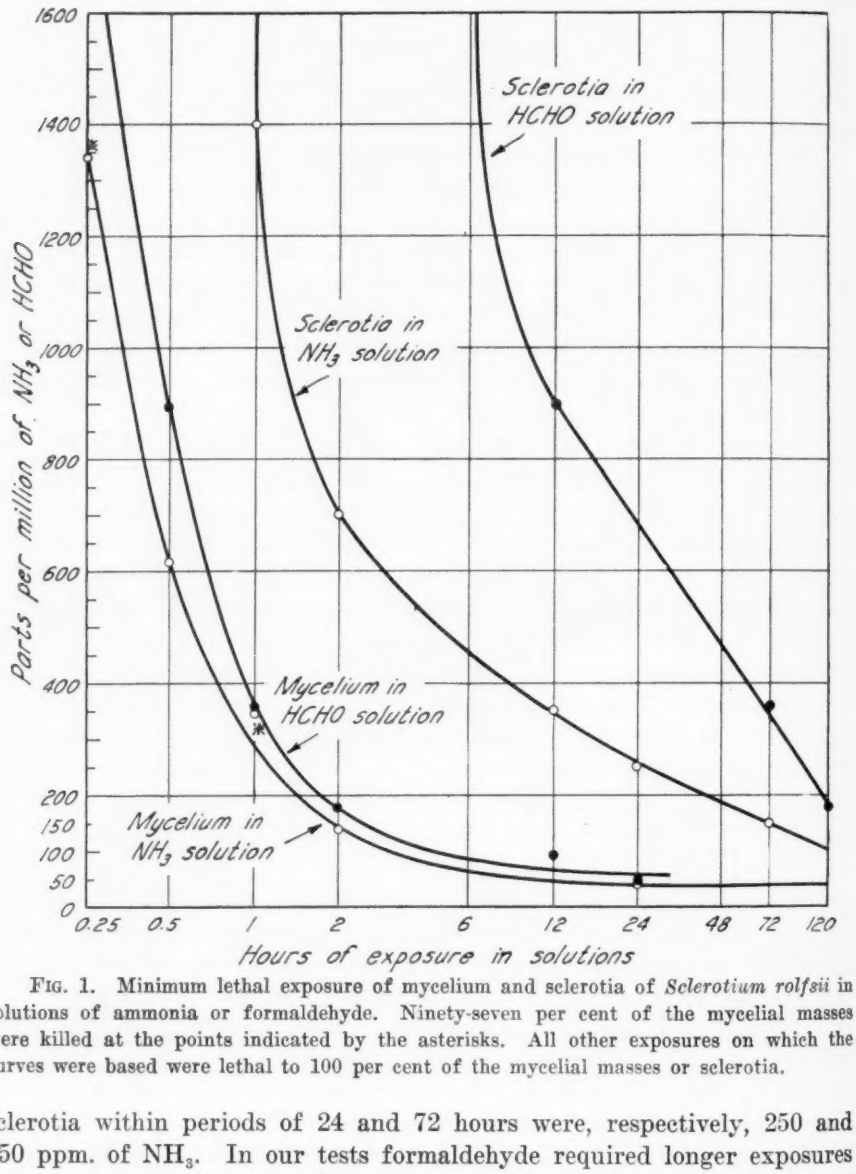

FIG. 1. Minimum lethal exposure of mycelium and sclerotia of *Sclerotium rolfsii* in solutions of ammonia or formaldehyde. Ninety-seven per cent of the mycelial masses were killed at the points indicated by the asterisks. All other exposures on which the curves were based were lethal to 100 per cent of the mycelial masses or sclerotia.

sclerotia within periods of 24 and 72 hours were, respectively, 250 and 150 ppm. of NH_3. In our tests formaldehyde required longer exposures than equal concentrations of ammonia to kill mycelium and sclerotia of *S. rolfsii*.

Previously, Neal[1,2] had found that mycelium of *Phymatotrichum omnivorum* was killed by 20 minutes' exposure in a solution containing 500 ppm. of NH_3. Sclerotia of the same fungus were killed by exposure for 20 minutes in 0.5 per cent of NH_4OH (approx. 2400 ppm. of NH_3).

The effectiveness of low concentrations of ammonia, as reported above, and the encouraging results of Streets[3] with heavy applications of ammonium sulphate and ammonium hydrate for the control of the cotton root-rot fungus on deciduous trees prompted applications of ammonium compounds on growing sugar beets in fields known to be heavily infested with *Sclerotium rolfsii*. Either anhydrous ammonia[4] or ammonium sulphate, dissolved in irrigation water in concentrations of approximately 300 ppm. of NH_3, significantly reduced the percentage of infection on sugar beets. The increase in yield of disease-free beets over nontreated plots was from 5 to 9 tons per acre and was more than sufficient to cover the cost of the treatments. Whether the beneficial results were due to the toxicity of low concentrations of ammonia in the soil or to the influence of the nitrogenous fertilizers upon the host plants has not been determined.—L. D. LEACH, AND A. E. DAVEY, Division of Plant Pathology, Branch of The College of Agriculture, Davis, California.

Blackberries: Possible Source of Streak Infection in Black Raspberries.—While making inspections during the summer of 1932 of bramble plantings entered for certification, 4 cases were found of a very severe streak infection on black raspberries when growing in close proximity to blackberries. In the literature on black-raspberry disease, nothing is found concerning the possibility of blackberries as a source of streak infection in black-raspberry plantings until Zundel[1] mentions it in 1934. Dodge and Wilcox,[2] in 1926, reported that streak may be found on blackberries, causing severe stunting. However, in neither case was any evidence presented to show the relationship between blackberries and black raspberries in the

[1] Neal, D. C., R. E. Wester and K. C. Gunn, Treatment of cotton root-rot with ammonia. Science n. s. 75: 139–140. 1932.

[2] Neal, David C., R. E. Wester, and Kenneth C. Gunn. Growth of the cotton root-rot fungus in synthetic media, and the toxic effect of ammonia on the fungus. Jour. Agr. Res. 47: 107–118. 1933.

[3] Streets, R. B. The treatment of deciduous fruit trees and nut trees infected by *Phymatotrichum omnivorum* with ammonium compounds. Science n s. 79: 417–418. 1934.

[4] Applied with apparatus furnished by and with the assistance of D. D. Waynick and F. H. Leavitt of the Association Laboratory, Anaheim, California.

[1] Zundel, George L., Raspberry diseases. Pennsylvania Agr. Exp. Sta. Ext. Circ. 133 (Revised), p. 18. 1934.

[2] Dodge, B. O., and R. B. Wilcox, Diseases of raspberries and blackberries. U. S. Dept. Agr. Farmers' Bul. 1488, p. 31. 1926.

spread of streak. Severe streak is characterized by a stunting of the growth, downward curling of the leaves, necrotic streaking of the new cane growth and production of a few seedy sour berries. On blackberries the symptoms become apparent during the latter part of May, often causing extreme stunting, but they usually do not appear on black raspberries until July.

Our records of Northwestern Pennsylvania show that in four cases in 1932, as well as one in 1933, blackberries were being grown adjacent to the black raspberries. Only mild infections of streak were found in any black raspberry plantings isolated 500 feet or more from blackberries.

One of the four cases mentioned as showing streaks is outlined in table 1. As will be noted, the first 2 rows are blackberries, while the balance of the planting is made up of black raspberries. In 1931 there was no streak found in either the blackberries or the black raspberries. In 1932, 1 per cent of streak was found in the blackberries and 30 to 60 per cent was found in the first two rows of black raspberries, 10 to 15 per cent in the next 2 rows, and 2 to 4 per cent in the last 2 rows. The plants for the Plum Farmer planting, set out in 1931, were taken from the planting set out in 1930. Plants from the 1930 Cumberland planting also were set out in 1931 on a neighboring farm, but were isolated from blackberries. This planting in 1932 showed one and one-half per cent of streak.

TABLE 1.—*Spread of streak disease in black raspberries beside blackberries on Young's Farm.*

	Variety	Per cent streak		
		1931	July 1 1932	August 1 1932
Rows 1 and 2, planted 1930	Eldorado blackberries	?	1	1
Rows 3 and 4, planted 1931	Plum Farmer black raspberries	0	30	60
Rows 5, 6, 7, planted 1930	Cumberland black raspberries	0	10	15
Rows 8, 9, 10, planted 1930	Plum Farmer black raspberries	0	2	4

Our records show that whenever a severe case of streak infection has been found in black raspberries, there always has been a planting of blackberries near-by. In one case we found the percentage of streak had increased from 6 per cent in 1931 to 30 per cent in 1932. In the adjacent blackberry planting there were a few black-raspberry mixtures that showed 100 per cent streak infection, while the blackberries themselves had approximately 1 per cent infection. On another farm, where black raspberries were being grown beside blackberries, the streak in 1931 was 5 per cent and in 1932 it had increased to approximately 75 per cent.

In another case there was a decided increase between the amount of

streak in black raspberries found in 1931 and that found in 1932. In this case no streak was found in the blackberries, and it could not be determined where the disease had its source. The percentage of streak found in black raspberries in all cases was always highest in the rows nearest the blackberries.

In 1933, a planting of black raspberries was found growing between two Eldorado blackberry plantings. All the black-raspberry plants were infected by streak but only one blackberry plant was suspected of being infected by streak.

Since no severe infection of streak was found in any black raspberry plantings isolated from blackberries and since the percentage of streak in black raspberries was always highest in rows nearest to blackberries, it is thought that, even in spite of the fact that blackberries often show little if any visible streak, there is a correlation involved that would bear deeper investigation.—J. K. THORNTON, State College, State College, Pennsylvania.

Susceptibility of Raspberry Species and Varieties to Leaf Spot (Mycosphaerella rubi) at Beltsville, Maryland.—The common leaf spot (*Mycosphaerella rubi* Roark) has been especially serious in a collection of raspberries at the U. S. Horticultural Field Station at Beltsville, Maryland, in 1934. Many varieties, including Viking, Lloyd George, and Newburgh, are not raised commercially so far south as Maryland because they are too susceptible to leaf spot to produce even a small crop there. The varieties represented in the collection at Beltsville are those that endure the conditions best and are actually somewhat resistant to leaf spot. However, such varieties as Latham and Chief were so badly affected that more than half their leaves were lost in 1934, and nearly all of the leaves of the variety June had dropped by the middle of September.

The relative resistance of the species and varieties is given in the following table. Some, listed in column 1 as more resistant than Latham and Chief, showed hardly a trace of leaf spot. In contrast, many varieties and a great many selections resulting from breeding work that are not listed in this table were more susceptible than Latham and Chief.

It should be noted that the 12 species listed are from eastern Asia and are highly resistant to leaf spot; in fact, no species from Asia so far tested has been found susceptible to leaf spot under conditions at Beltsville. It is of interest, also, that most Asiatic species tested are immune from or highly resistant to anthracnose and spur blight. There are no standard red or black raspberry varieties among those listed in column 1 as most resistant, but there are several little-known varieties of both in this list. It seems evident that resistance may be obtained both through breeding within the species, as indicated by breeding these resistant American varieties, as well as by hybridizing American with Asiatic species.

Hardiness of American varieties in Maryland is largely dependent upon resistance to leaf spot. When leaf spot is epidemic and the canes nearly defoliated as early as August or September, food reserves are not accumulated in sufficient quantity to make the plants hardy and the canes are more

Susceptibility of raspberries to leaf spot at Beltsville, Maryland, September 24, 1934

More resistant than Latham	As resistant as Latham and Chief	Highly susceptible, more than Latham
Rubus biflorus	Bristol-black	Black Beauty-black
" *incisus*	Dundee-black	Black Giant-black
" *innominatus*	Golden King-black	Cumberland-black
" *inopertus*	Naples-black	Variety from Kansas-black
" *Kuntzeanus*	Potomac-purple	Farmer-black
" *mesogaeus*	U.S.D.A. 5, 7-red	Honeysweet-black
" *parvifolius*	N.Y. 5371-red	June-red
" *niveus*	N.Y. 6523-red	Madison-black
" *phoenicolasius*	N.Y. 8126-red	New Logan-black
" *rosaefolius*	N.Y. 14443-red	Perfection-black
" *veitchii*		Quillen-black
" sp. F.P.I. 69115		Royal-purple
Van Fleet (*Kuntzeanus* × *Cuthbert*)		White Queen-red
Variety from Arkansas-red		
F.P.I. 75145-red		
U.S.D.A 0, 8, 9, 11-red[1]		
N.Y. 2609-black[2]		
N.Y. 5555-red		
N.Y. 5621-red		
N.Y. 5670-red		
N.Y. 10723-purple[3]		
N.Y 10724		
N.Y. 14619-red		

[1] Red = derived from *R. strigosus, R. idaeus,* or hybrids of these two species.

[2] Black = *R. occidentalis.*

[3] Purple = hybrids of red and black varieties.

readily injured by winter cold. Leaf spot was epidemic in 1933 as well as in 1934, and it is for this reason that in most sections east of the Allegheny Mountains Latham showed severe winter injury last spring from central New Jersey southward, wherever this disease was not controlled by spraying.—GEORGE M. DARROW, Bureau of Plant Industry, U. S. Dept. of Agr. Washington, D. C.

Sclerotinia Wilt of Canada Thistle.—During the summer of 1932 attention was called to the dying of Canada thistle (*Carduus arvensis* (L.) Robs.) near Craig, Colorado, and several large patches of the thistle were kept under observation.

A fungus was consistently isolated from the diseased plants and identified as *Sclerotinia sclerotiorum* (Lib.) Massee, after making host-range tests and comparing with a known culture of *S. sclerotiorum*. Both cultures reacted similarly on potato, tomato, bean, lettuce, celery, sunflower, and Canada thistle plants. Working with the same fungus, Young and Morris[1] observed that Canada thistles commonly were killed when growing close to wilting sunflowers.

Canada thistle plants completely wilted within 5 days after inoculation with the fungus. The cankers on the roots have a rather soft consistency, gray to dark brown, and of a water-soaked appearance. The parenchyma tissue in the diseased area is decomposed, leaving only the lignified tissues

FIG. 1. Part of a large patch of Canada thistle growing near Craig, Colorado, affected with *Sclerotinia sclerotiorum*. The advancing outer edge of the patch is seen to the left and the dying central area to the right.

[1] Young, P. A., and H. E. Morris. Sclerotinia wilt of sunflowers. Montana Agr. Exp. Sta. Bul. 208. 1927.

and giving the root a shredded appearance. Irregular, hard, black sclerotia are produced both on the outside and in the cavities of the diseased stem.

Because of the rapidity and severity of attack, use of the fungus was suggested as a possible means of controlling the thistle. By the summer of 1934, however, it was found that the organism did not completely destroy the thistle. Though large patches were nearly destroyed, a vigorous fringe of the thistle kept ahead of the fungus and a "fairy ring" type of growth resulted (Fig. 1). As *Sclerotinia sclerotiorum* is not completely effective and because of its wide host range, its use as a biologic control agent is out of consideration.—E. W. BODINE, Colorado Agricultural Experiment Station, Fort Collins, Colorado.

REPORT OF THE 1935 ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The annual meeting of the Southern Division of The American Phytopathological Society was held in the Henry Grady Hotel, Atlanta, Georgia, January 31–February 1, 1935, in conjunction with the Association of Southern Agricultural Workers.

The attendance was unusually large, there being from 30 to 35 present at each of the 3, half-day sessions, and among these were pathologists from 10 States.

At a business meeting the following officers were elected:

President—G. F. Weber, University of Florida, Agricultural Experiment Station, Gainesville, Florida

Secretary-Treasurer—J. C. McKee, Mississippi State College, State College, Mississippi

Councillor—(reelected) V. H. Young, University of Arkansas, Agricultural Experiment Station, Fayetteville, Arkansas

Thirty-five papers were presented. Twelve papers dealt with diseases of fruit and vegetable crops, 11 with cotton diseases, and 12 with diseases of miscellaneous crops. January 31 was devoted to discussions of cotton in its various aspects by the different Sections comprising the Association, and on that day the phytopathologists held a symposium on cotton-disease problems that occupied a morning session. Representatives were present from most of the important cotton-producing States and an interesting and mutually helpful discussion of the cotton-disease problems was given.

Other papers were those relating to diseases of strawberries, vegetable crops, and tobacco.

Dinners and entertainment, which were thoroughly enjoyed by all who attended, were given for the Association on January 30th by the Chilean Nitrate Educational Bureau, Inc., and by the Agricultural Development Bureau of the Barrett Company on January 31st.

Eugene C. Tims, *Secretary-Treasurer*

A Rhizoctonia Bud Rot of Strawberry.—A. N. Brooks.

Rhizoctonia bud rot of strawberries in Florida occurs during the cool, moist weather of November to March. Although not widespread, and, therefore, of minor importance, it does cause severe damage in individual fields. The disease symptoms are dry rots of leaf- and flower-buds, and bases of petioles and stipules, the last causing the older leaves to lose their upright position and lie flat on the ground. Death of affected plants sometimes results. Recovery takes place through the development of adventitious buds. Under favorable conditions of low temperature and abundant moisture any aboveground plant part in contact with the soil may develop the rot. Fruit in

any stage of maturity is subject to this rot, which is probably the same fruit rot described by Dodge and Stevens. The roots and rhizomes are not affected. The causal organism belongs to the group represented by *Rhizoctonia solani*. Healthy plants in sterile soil readily become diseased when inoculated with sclerotia of this rhizoctonia either by placing the sclerotia in the buds or in the soil. The environmental conditions necessary for the rapid development of bud rot are temperatures below 75° F. and relatively high humidities, together with high organic soil content. Spraying with Bordeaux mixture is ineffective and likewise undesirable because of the fruit on the plants at the time the disease develops. The possible means of control seem to be (1) lowering of the organic content of the soil by reduction in summer cover crops; and (2) supplying adequate air drainage about the plants by greater spacing of the plants on the beds, or by later setting of plants so as to maintain a bush of smaller size.

Sclerotium rolfsii Sacc. on Strawberries and the Effect of Certain Chemicals on the Sclerotia.—R. E. NOLAN.

During the past 3 years *Sclerotium rolfsii* has occurred on strawberry plants in the Plant City area, especially during the warm months. All parts of the plant may be attacked, although often only one part, the bud, the fruit, or the roots, is found severely infected. The lower leaves of infected plants lie close to the ground. The lower part of the petiole and stipules generally shows the first signs of infection. The fungus later invades the bud, quickly killing it. Adventitious buds may or may not be formed later. Occasionally, during wet periods in the spring, newly set plants are attacked. The root systems of such plants are covered with a mass of white mycelium, and the roots are light brown. After death, sclerotia sometimes form. During the fruiting season an irregular, light brown soft spot may be found on the soil side of some of the small berries. As the berry grows the spot becomes larger, until ripening occurs. An abundant quantity of the white mycelium is then produced, completely covering the berry and causing a soft rot. When the berry is consumed the mycelium disappears, leaving a mass of sclerotia and strawberry seed on the surface of the soil.

Inoculation experiments show that a saturated atmosphere quickens the germination of the sclerotia and subsequent infection. In tests conducted to date to determine the effect, formaldehyde and carbon bisulphide killed all sclerotia, while benzol, paradichloralbenzene, chloroform, ether, and ammonia, as well as xylol, permitted varying amounts of germination.

A Bark Disease of Tahiti Lime Trees Caused by Phomopsis citri Fawcett and Diplodia natalensis Evans.—W. B. TISDALE.

Tahiti lime trees planted in Florida often die during the first 4 years after they are set in groves. Some trees die back quickly from the pruning cuts; others make vigorous growth for 2 or 3 years before they show signs of disease. The decline is often chronic. Infection of larger trees is manifested by a yellowing of the foliage and dead areas of bark on the trunk or large branches. The wood under the dead bark also is invaded and is usually darker than normal. Gum frequently exudes from the invaded areas. Salmon pink spore masses of a Fusarium and pycnidia of a Diplodia are frequently observed on dead areas on the trunks of large trees.

Several fungi have been isolated from the invaded parts of trees of different ages, but *Phomopsis citri* and *Diplodia natalensis* have been obtained singly or in combination in a majority of cases. Phomopsis occurs more frequently in diseased young trees and small branches, while Diplodia is associated more frequently with affected

trunks and large branches of older trees. Both organisms have been highly pathogenic in inoculation tests. Only a few of the other organisms isolated have shown any signs of parasitism under similar conditions.

Anthracnose Disease of Eggplants.—T. D. PERSONS.

On October 25, 1934, specimens of eggplant fruits showing a fruit rot were received from Sanatorium, Mississippi. One fruit showed 2 different types of lesions and examination with the microscope revealed that 2 different fungi were responsible. The fungus in one of the spots rather closely resembled the description given of *Gleosporium melongenea*. The fungus found in the other was entirely different as to spore size and shape. Cultures of this fungus were made and it was found to resemble closely a culture of a Collectotrichum isolated from Lima beans some time ago. The Lima bean fungus recently has been referred to *Colletotrichum truncatum* (Schw.), n. comb., by C. F. Andrus of the U. S. Department of Agriculture. A review of the literature on eggplant diseases has failed to disclose a species of Colletotrichum of this description on eggplant. Accordingly, preliminary inoculation experiments were begun in order to determine whether the eggplant fungus was the same as the bean fungus. Readings were taken about 10 days later and it was found that the lesions produced on the young bean stems by the eggplant fungus were identical with those produced by the bean fungus, *Colletotrichum truncatum* (Schw.), n. comb.

Only Certain Strains of Tobacco Mosaic Cause Mosaic Burn.—W. D. VALLEAU AND E. M. JOHNSON.

Attempts to produce mosaic burn experimentally, using known strains of the virus, demonstrated that certain strains are entirely incapable of causing the disease. Inoculations made from mosaic-burned plants in the field regularly produced burn in the field and sometimes in the greenhouse. Dried specimens, collected 8 years ago from burned plants, still retained the ability to cause burning. If the finger method is used, necrosis typical of mosaic burn develops at the points of inoculation with the burn virus, but not with the nonburning strains. The local spots are distinct from the type of spots that occur on inoculated leaves of *Nicotiana glutinosa*. Inoculation of White Burley tobacco with a mosaic-burn virus at topping time has a tendency to increase the value of the upper leaves, changing the burned leaves from red tips to a smoker grade. The burned-tip leaves have much the appearance of flyings.

Further Studies on the Effect of Ammonia Nitrogen on Growth of the Cotton-root-rot Fungus, Phymatotrichum omnivorum, in Field and Laboratory Experiments.—D. C. NEAL.

Experiments conducted at Greenville, Texas, for the past 4 years have shown that the cotton-root-rot fungus, *Phymatotrichum omnivorum*, is highly sensitive to ammonia and ammonium compounds. In previous nutrition studies, it was found that ammonia nitrogen, when used in Duggar's solution at concentrations to yield 12.4 or 6.2 grams, respectively, of N per litre, has a toxic effect on the fungus, whereas similar concentrations of nitrate nitrogen produce an abundant growth. Additional experiments conducted in 1934, but with the nitrogen content still further reduced (supplied to cultures to yield 2.8 grams per litre), have largely confirmed the results of previous studies. No growth of mycelium occurred after 15, 30, 45, and 60 days with ammonium phosphate or ammonium sulphate; scant growth appeared with ammonium nitrate and di-ammonium phosphate after 15 and 30 days; and moderate growth after 45 and 60 days. In the

nitrate-nitrogen group, the best growth occurred, as heretofore, when calcium nitrate was supplied as the N source.

Sclerotia and hyphal strands formed only in cultures supplied with calcium nitrate and di-ammonium phosphate.

Concentrations of 50 p.p.m. NH_3 killed the root-rot mycelium in sectional-agar blocks in 24 hours, and concentrations of 300 p.p.m. killed the sclerotia in 1 hour. The residual effects of field applications of ammonium compounds made in 1932 were still marked in 1934 in both plot and barrier treatments, and show that the use of ammonia as the N source for certain Texas blackland soils may be a means of avoiding serious losses from root rot.

Control of Diseases of Tomato Seedlings.—FRANK VAN HALTERN.

During 4 years' cooperative tomato seed treatment and seed-bed spraying tests with 4 northern experiment stations have revealed that copper-lime dust did not control Macrosporium blight, OXO Bordeaux gave good disease control, and homemade Bordeaux mixture gave best results. The latter, combined with seed treatment, controlled Macrosporium and bacterial spot while without seed treatment control was fair.

Heavy seed dusting with Semesan Jr. reduced the stand but controlled Macrosporium blight, Semesan failed to control, and a 5 minute 1–1000 mecuric chloride solution controlled Macrosporium blight and bacterial spot. Monohydrated copper sulphate dust caused severe injury in dry, sandy soils.

Macrosporium overwintered in the soil but in two tests bacterial-canker failed to overwinter. Bacterial-canker was held down to a small amount by a seed treatment and spray combination.

Recommendations suggest state certified seed, a 5-minute treatment in a 1–3000 mercuric chloride solution, crop rotation, and spraying with 2–3–50 Bordeaux mixture when the second rough leaf appears followed by 3–4–50 sprays at intervals not exceeding 2 weeks.

Do Necrotic Lesions Result in Localization of Tobacco-Mosaic Viruses in Nicotiana?—W. D. VALLEAU.

Studies originally designed to put into practice the control of tobacco mosaic through localization of the virus in necrotic spots have demonstrated that necrotic spotting around the points of entrance of the virus and failure to become systemic are not correlated in species of Nicotiana. Seven strains of mosaic have been found that cause local necrotic spotting in several varieties of *N. tabacum* but not in others. In each variety these viruses became systemic. The factor for necrotic spotting in *N. tabacum* is a Mendelian dominant. Plants of *Nicotiana acuminata, N. langsdorffii, N. sanderae* and *N. rustica* inoculated each with one of several strains of tobacco mosaic developed necrotic spotting, but this did not localize the virus. In each species the virus spreads slowly from the leaf into the stalk, sometimes becoming systemic in *N. langsdorffii* and *N. rustica*, partially systemic in *N. sanderae*, and causing stalk necrosis and streak in *N. acuminata*. In *N. tabacum* var. *Ambalema*, necrotic spot-producing viruses failed to become systemic, probably because of resistance in this variety rather than because of necrotic spotting. With the possible exception, of Ambalema, necrotic spotting appears to be associated with a high degree of sensitivity to the virus resulting in extensive necrosis as the virus spreads.

The Etiology of Damping Off of Cotton Seedlings.—C. H. ARNDT.

Glomerella gossypii caused heavy losses of cotton seedlings in South Carolina in 1934. Greenhouse studies have shown that this fungus may cause damping off at soil temperatures

as high as 30° C. Other parasites that have been isolated from diseased hypocotyls of cotton in South Carolina are: 1. Nematodes. Several species of free living nematodes frequently invade normal hypocotyls. They apparently do not produce damping off when not associated with bacteria and fungi. 2. Fusarium spp. Fifteen to 75 per cent of the isolates from diseased hypocotyls have been species of Fusarium. Those which have been found sufficiently virulent to cause damping off have not been isolated frequently enough to indicate that they are a significant cause of losses of stand. 3. *Pythium ultimum* was found to be a frequent cause of poor germination and loss of stand in one field. 4. Bacteria. All attempts to produce typical damping off with the bacteria isolated from diseased hypocotyls have been unsuccessful. 5. Rhizoctonia. This fungus has been found on less than 10 per cent of the diseased hypocotyls examined during the past 10 years. These results do not support the generally accepted belief that Rhizoctonia is the usual cause of the damping off of cotton seedlings.

Control of Potash Hunger and Fusarium Wilt in Cotton.—V. H. YOUNG, J. O. WARE, and O. A. POPE.

Studies with wilt resistant varieties of cotton in western Arkansas in 1934 gave results comparable with those secured during 1927–1934 in eastern Arkansas. Of the 18 varieties studied, five showed from 4 to 8 per cent of cotton wilt compared to 29 to 44 per cent for the 5 most susceptible varieties. Highly resistant varieties were Clevewilt 33–6, Dixie 14, Rhyne's Clevewilt 33–10, Coker's Clevewilt 3 and Rhyne's Clevewilt. Highly susceptible varieties were Startex, Acala 1114, Super Cleveland, Trice 304, and Half and Half In eastern Arkansas where 36 varieties were studied, the incidence of cotton wilt ranged from about 1 per cent in Rhyne's Clevewilt to 49 per cent in Half and Half. In most cases a low incidence of cotton wilt was correlated with high yields and those varieties most severely attacked were invariably in the low-yielding group.

Five years' work indicates that the control of potash hunger or "rust" through the use of potash-containing fertilizers results, under central and eastern Arkansas conditions, in a marked decrease in the severity of cotton-wilt attacks. In 1933 and 1934 the use of a resistant variety of cotton (Rowden 2088) and potash fertilization gave the best control of "rust" and cotton wilt. On potash-depleted soils in Arkansas the control of potash hunger and wilt through potash fertilization and a suitable wilt-resistant variety of cotton proved to be the best control for these troubles available.

Seeds of Watermelons and Okra as Possible Carriers of Fusarium Wilt.—By J. J. TAUBENHAUS.

Extensive cultures were made of the roots, stems, pods, or rinds, and seed of watermelon and okra plants infected, respectively, by *Fusarium niveum* and *F. vasinfectum*. *F. niveum* was recovered from all parts of the roots, stems, peduncles, rind, and from only a very small percentage of the interior of the seed of fruit from infected watermelon plants cultured. Similar results were obtained with okra, namely, *F. vasinfectum* was recovered from all parts of the roots, stems, pod tissue, and from only a very small percentage of the interior of the seed from the pods of such plants. These results indicate that the seeds from either infected watermelons or okra may act as internal carriers of fusarium wilt.

On a Black Crown Rot of Greenhouse Snapdragons Caused by Myrothecium roridum Tode.—By J. J. TAUBENHAUS.

During the fall and winter of 1933 and 1934, snapdragons in a commercial greenhouse at Waco, Texas, were dying from a crown rot that killed over 90 per cent of the plants.

The affected crowns are at first water-soaked, then shrink, dry, and become covered with a thin white mycelium and numerous black sporodochia; finally, the epidermis and cambium crack open and become shredded. A fungus, *Myrothecium roridum*, was recovered from the infected tissue. The organism was successfully used to inoculate normal plants in which the disease with its typical symptoms subsequently developed.

Mode of Action of Bordeaux on Mycosphaerella fragariae.—A. G. PLAKIDAS.

Previous studies had shown: (1) That infection with the leaf-spot fungus obtains primarily through the dorsal surface of the leaf; and (2) that the disease can be controlled effectively by spraying with Bordeaux, even though very little of the spray reach the dorsal side of leaf. The present study has shown that Bordeaux is not protective in the sense that it protects the sound sprayed leaves by killing the sports that fall on them, thus preventing infection, but rather that Bordeaux affects the inoculum at its source. The spray does not penetrate to kill the mycelium in the tissue, but it greatly inhibits spore production on the sprayed spotted leaves. Comparative spore counts from sprayed and nonsprayed leaves showed that between 6 and 10 times as many spores were produced on the latter as on the former. Bordeaux is lethal to the nongerminated spores, even in relatively high dilutions. One hour's exposure to 1–21 dilution of a 4–4–50 Bordeaux mixture resulted in 100 per cent killing of the spores.

A Resumé of Cottonseed Treatments in South Carolina.—C. H. ARNDT.

A resumé was given of the effect of cottonseed treatments with dusts of the type of Ceresan on germination and yields in South Carolina since 1929. The data as presented were a composite of the results of W. D. Moore, 1929–30; E. E. Hall at the Pee Dee Experiment Station 1930–34; and for the Clemson College Experiment Station 1931–34. Treating natural seed increased the average stand of seedlings 18 per cent and increased the average yield 5.7 per cent. Acid delinting of the seed increased the average stand 50 per cent and the average yield 8.5 per cent as compared to natural, linted seed. Treating acid-delinted seed increased the average stand 25 per cent as compared to nontreated delinted seed, but did not increase the average yield. Machine-delinted seed gave an average increase of 80 per cent in stand, and increased the average yield 1 per cent in tests over a 3-year period as compared to natural seed. Treating machine-delinted seed gave an additional average increase in stands of 35 per cent, and 5 per cent in the average yields. Dr. Moore's results show significant reductions in the amount of angular leaf spot when treated seed was used.

The Reaction of Several Isolations of the Cotton-Wilt Fungus to Toxic Dyes.—GEO. M. ARMSTRONG AND C. N. CLAYTON.

One of the best of wilt-resistant cottons at Hartsville, South Carolina, wilted badly at Manning, S. C., in 1931. Cut stems of this variety from Manning showed a blackened ring in the center, while most of those plants from Hartsville showed blackening chiefly in the outer edge of the fibrovascular bundles. White and lilac to rose purple cultures were obtained. An attempt to differentiate between probable strains of the fungus, *Fusarium vasinfectum*, by use of 4 cotton varieties grown in the greenhouse was inconclusive. Coons and Strong's method of diagnosing species of Fusarium by means of growth-inhibiting substances in a synthetic culture, using malachite green and crystal violet, was then tried. Three dye tests have been made, with 8 cultures from different sources, noting color, size of colony, tolerance for toxic dye, and general growth characters. There were changes in color production by the same strain from test to test, and also changes in tolerance for the toxic dyes. The changes in color, growth, and tolerance for toxic dyes are in keeping

with the changes in various Fusarium species, as reported by Leonian and others. Our results with *F. vasinfectum* also are similar to those of Sleeth with *F. niveum*, in that a classification of "strains" made at one time will not be the same as that made at another time. A field test with these isolates is desirable, but there has been no opportunity to test them.

Studies on Downy Mildew of Tobacco.—G. M. ARMSTRONG AND C. B. SUMNER.

Three methods of control of tobacco downy mildew have been tried: sprays, high temperature, and forced air ventilation. Colloidal copper and Cal-Mo-Sul sprays saved many small plants. If the disease was delayed until plants were almost ready to set in the field, the sprays were of no value since practically all plants, regardless of treatment, became infected and few plants were killed even without the sprays. Cal-Mo-Sul, in 1934, gave a harmful residual effect in the soil. Five experiments on control by high temperatures, conducted in well-built beds, covered either by glass, Cel-O-Glass, Vio-Ray, Flex-O-Glass, or cloth, and heated by either electric light bulbs, G. E. heating cables, kerosene lanterns, or hot water in pipes heated by a kerosene heater, have shown minimum temperatures of 34° C. and 31° C. controlled the disease, but a minimum temperature of 29° C. or lower gave unsatisfactory control. Glass sash was superior to the other cover materials tried. Kerosene lanterns proved impracticable. In two instances, air heated by charcoal fires was conducted through flues either underground or along the inside of the beds. High temperatures were not maintained throughout the night. Outside air forced over a heating device into the bed protected fairly large plants but smaller ones near the soil were killed.

Dissemination of the Bacterial Leaf Spot Organism.—F. M. ROLFS.

The causal organism, *Bacterium malvacearum*, is carried from crop to crop on the seed. Seed is the source of both primary and secondary infection. There are two means of internal seed infection: Direct, by an invasion while the boll is quite young; indirect, by contaminated water through a break in outer seed coat of mature seed at the micropyle.

Water is the most important agent of dissemination. The bacteria were carried 980 feet in rill water. The green leaf bacteria have an independent movement in still water of 12 inches or more. 60 hours is the approximate age limit of the free bacteria in distilled water. Soluble soil salts in contaminated pool water shorten the life of the bacteria. Hot, dry weather tends to eliminate the more exposed parenchyma infections. The leaf spots become less angular in form, and the vascular invasion develops more prominently. The massing of the bacteria in veins and midrib hastens the shedding of the leaves, and affords better protection for the parasite from adverse weather conditions.

Wind is the most active agent of dissemination. A single whirlwind scattered the infected dry leaf material over an area of 100 acres in 20 minutes.

Breeding Peanuts for Disease Resistance.—B. B. HIGGINS.

Peanut breeding by the writer was started in 1931, with the objective of obtaining disease-resistant varieties better suited to southern Georgia conditions than those now grown. Approximately 200 crosses have been made, including various combinations between 15 varieties, each of which possessed some desirable characteristics. Complete records were kept of the principal characteristics of each progeny plant—about 200 first. Preliminary study of the data collected indicated that in most of the characters under consideration the first-generation plants are intermediate between the 2 parent varieties. In the second generation they break up in approximate Mendelian ratios. Resistance to the 2 leaf spots (*Cercospora personata* and *C. arachadis*) is apparently inherited independently. Selections, very resistant to one, are often extremely susceptible to the other.

No exact data have been obtainable on the inheritance of resistance to *Sclerotium rolfsii;* but we find some hybrid strains extremely susceptible and others quite resistant.

Results from Ten Years' Work on Cotton Seed Treatment.—B. B. HIGGINS.

In a more than 10-year field study of the efficiency of various disinfectant treatments for cotton seed some have given perfect control of angular leaf spot and considerable reduction of seedling anthracnose. Our records show small but consistent yield increases from the better dust treatments, even when planted in plats alternated with plats or rows planted with nontreated seed. Apparently, this increase was correlated with the better stand obtained with treated seed. In isolated walled beds constructed in a field where cotton had not been grown for 25 years, treated seed gave 1½ to 4 times the yield obtained from the check bed with an equal number of plants from nontreated seed, the first 3 years. The fourth year 100 lbs. of cotton seed was worked into the soil of each bed during the winter in order to get the soil evenly infested with the soil organisms usually carried on seed, especially *Fusarium moniliforme.* During the following 3 years the yields from the check bed have approximately equaled those from beds planted with treated seed.

The results indicate that disinfection of cotton seed may have considerable value for seed of low germination, such as that saved during a rainy season, and especially valuable when planting on land not devoted to cotton for several years.

Report of One Year's Test of Copper Fungicides and Other New Materials as Peach Sprays.—L. E. MILES.

Two applications of each of the following sprays were made on plats consisting of 5 trees for each of the treatments: (1) Self-boiled lime sulphur, 8–8–50; (2) wettable sulphur, 4 pounds, water, 50 gallons; (3) lime-sulphur concentrate, 1 gallon, Precipto, 1 quart, lime, 4 pounds, water, 50 gallons; (4) copper silicate, 2 pounds, Bentonite, 2 pounds, lime, 4 pounds, water, 50 gallons; (5) copper oxide, 2 pounds, Bentonite, 2 pounds, lime, 4 pounds, water, 50 gallons. Sprays were applied May 11 and May 30.

Brown-rot control was almost complete with all treatments. In no case was scab serious enough to affect the keeping quality of the fruit or to influence its market value except on a very discriminating market. The copper silicate treatment showed the greatest amount of both scab and spray burn with the copper oxide treatment next. The lime-sulphur concentrate and Precipto spray caused some spray injury both on foliage and fruit, but it was of negligible importance. Self-boiled lime sulphur gave best results in all cases.

Tobacco Bed Soil Disinfection.—J. O. ANDES.

Tests conducted over three reasons for tobacco bed disinfection indicate that a 3 per cent ammonium thiocyanate solution applied at the rate of 2 quarts per square foot, apparently disinfects the soil satisfactorily, judging by the killing of common weed seeds. The application should be made six weeks before seeding in order for it to disintegrate and become nontoxic to the tobacco seed.

The Verticillium Wilt Disease of Cotton.—L. E. MILES.

This disease was first reported in Mississippi in the fall of 1930. It has been found in 9 counties in the State and reported from Tennessee, Arkansas, California, Oklahoma, on 2 plants in Virginia, and from Greece. In 1933 field inoculations were made in July with strains of Verticillium (1) from cotton at Stoneville, Mississippi, (2) from cotton in California, and (3) from Irish potatoes in St. Catherines, Canada. The strain from Mississippi produced evident infection within 10 days. In 2 weeks 100 per cent of the

inoculated plants were severely infected and wilted. The strain from Canada produced no infection. That from California caused infection on 16 per cent of the inoculated plants, though the symptoms never were so pronounced nor the injury so great as from the Mississippi strain. The California strain caused no mottling or blotching, though those symptoms were pronounced on plants infected with the Mississippi strain. Similar inoculations identically made during hot, dry weather of August gave no infection with either strain. Potted greenhouse tomatoes were inoculated with the same strains in the spring of 1933. The Canadian strain caused much severer and earlier symptoms than did either of the others. Very little difference was observed in the effect produced by the Mississippi and California strains. Inoculations on eggplants, snapdragons, beets, and California poppy showed the Mississippi and California strains much more virulent than the fungus from Canada. The latter caused no infection on California poppy. The California strain also differs from the others in morphology of the fungus in culture. It produces no microsclerotia, whereas the others do.

Angular Leaf Spot of Cotton in Mississippi in 1934.—L. E. MILES.

A serious epiphytotic of angular leaf spot appeared on cotton in the Yazoo-Mississippi Delta section of Mississippi following a tropical storm that swept that area on June 16 and 17. The trouble was prevalent also in some other parts of the State, but nowhere to the same extent as in the above area. The heavily infested district comprises the 13 northwestern counties, in which the greater part of the cotton of the State is grown. Spread of the trouble was directly attributable to wind-blown rain. A survey made in early July showed very severe defoliation and shedding of squares and young bolls. In many fields 100 per cent of the plants were affected and 60 per cent of the foliage was shed, the fallen leaves completely covering the ground between the rows. In such fields, where dry weather throughout the season followed, secondary infection was limited but the plants and bolls remained small and developed abnormally. Where rains occurred at intervals during the season secondary spread to leaves and young bolls was heavy. Counts during the fall and winter showed abnormally low germination throughout the entire area in most cases, probably directly due to angular leaf spot subsequent to the heavy defoliation and, especially, in the drier areas, to the failure of the bolls to develop to full size and maturity.

With the opening of the bolls, heavy seed infection occurred from bacteria dripping in rain and dew from boll and foliage lesions onto the exposed lint. Samples of seed taken at random from the infested district have produced a high percentage of infected seedlings.

Efforts have been made to have as much seed acid-delinted or treated with 2 per cent Ceresan dust as possible to secure protection of the new crop.

Anthracnose and Wilt of Strawberry Caused by Colletotrichum fragariae.—A. N. BROOKS

Losses from anthracnose or runner spot and wilt have been very high during certain summers in the central Florida strawberry area. Girdling of runners prevents development of new plants, and rotting of rhizomes results in wilting and death of plants that have become established. The disease symptoms are (1) typical anthracnose-like spots on runners and petioles and (2) reddish brown, dry rot of rhizomes, which causes wilting and death of plants. The causal organism is *Colletotrichum fragariae*. Environmental factors that favor rapid development of the disease are temperatures above 75° F. and high atmospheric humidity and soil moisture. Various spray mixtures and dusts have been used during the past 2 summers in experiments attempting to control the disease. Under periods of frequent rainfall all of the materials have failed to control, but, under

somewhat drier conditions, a pine oil containing 15 per cent copper resinate gave the highest percentage of control.

Wilt-resistant Cottons Adapted to the Gulf Coastal Plains.—D. C. NEAL.

In experiments conducted in cooperation with the South Mississippi Branch Experiment Station, at Poplarville, from 1932 to 1934, inclusive, the most adaptable and wilt-resistant cottons for that area have been selections of Dixie-Triumph, Miller, and D. & P. L. With these cottons, yields in excess of a bale per acre have been made under conditions of severe wilt infestation. Also, several selections have yielded a good quality of fiber of the desired length, ranging, in the case of the Dixie-Triumph, from 1 to 1 1/16 inches and from 15/16 to 1 inch in the case of the Miller. These cottons also are vigorous and apparently more resistant to leaf-spots than other varieties.

Other cottons that also have exhibited some degree of wilt-resistance in these tests, but are less adapted to the above area, are selections of Acala, Cook, Toole, Nucala, Clevewilt, Super Seven, Lightning Express, and Mexican Big Boll.

The Direct Effect of Bordeaux Mixture on Early Cucumber Production.—GEORGE F. WEBER.

Studies during the past season at Gainesville, Florida, have indicated that cucumber plants infected with downy mildew show increase in yield from the application of 2–4–50 Bordeaux mixture only when the picking season continues for more than 30 days.

Flower and fruit production, determined by daily counts throughout the season on 66 sprayed plants and 66 nonsprayed plants, showed that the nonsprayed ones produced more staminate and pistillate flowers and set more fruit each week for 3 consecutive weeks from the beginning of the season than did the sprayed plants. During the fourth week and thereafter, the sprayed plants exceeded the nonsprayed plants in flower and fruit production, because the latter were more than half defoliated by the disease at the end of the third week.

Sclerotinia Rot of Irish Potatoes in Florida.—A. H. EDDINS.

Sclerotinia rot of Irish potatoes caused by *Sclerotinia sclerotiorum* (Lib.) Mass., occurs generally throughout the Hastings potato area where, in 1933 and 1934, it was epidemic in a few fields. The fungus attacks the main stem, branches, and leaves of the potato plant. The tubers are not invaded but their growth is checked when the top of the plant is damaged by the disease. Epidemics of the disease occur on low ground during cloudy, foggy, and rainy weather. In 1934 70 per cent of the plants growing in the low areas in one field were killed by sclerotinia rot. Maximum yield reductions result when the main stems of the plants are invaded by the fungus 30 or more days prior to digging.

A Root Rot of Strawberry Caused by a Species of Diplodia.—R. E. NOLAN.

A quick-killing root rot of strawberry has been found to be caused by a species of Diplodia. The organism is readily isolated and inoculation experiments have proved it to be the cause of the disease. The disease is first noticed as a small dark brown spot on the roots, which quickly rots the entire root. Sometimes the affected root breaks off near the origin of infection, but the disease continues up to the plant. Inoculated plants are killed in from 6 to 10 days during hot weather. Soil-temperature experiments show that it is most destructive when the temperature is above 80° F. The organism belongs to the "natalensis" group of the genus Diplodia. Comparison with several other species of this group shows that it has characteristics common to some of them and varies in other characters. Cross-inoculation experiments with the several species also gave variable re-

sults. It is more active than *D. frumenti* in infecting corn, while, of the species used in comparison, *D. natalensis* attacked the strawberry more readily than any of the others.

Soil-temperature Studies on Florida Cigar-wrapper Tobacco.—R. R. KINCAID AND L. O. GRATZ.

Black shank (*Phytophthora parasitica* var. *nicotianae* Tucker) is a most important disease of Florida cigar-wrapper tobacco. Extensive experiments under controlled conditions of soil temperature and known soil moisture were conducted for the purpose of establishing the cardinal soil temperatures for the growth of transplanted tobacco seedlings, as well as for the development of the disease. Resistant No. 301 and susceptible Round Tip tobacco were used in these experiments. The cardinal temperatures for the growth of transplanted seedlings were found to be 9°, 28°, and 40° C. The maximum for the development of black shank varied from 16° or lower for newly transplanted seedlings to about 24° for plants inoculated several weeks after transplanting; the optimum and the maximum were about 28° and 34°, respectively. Some field observations indicated that soil temperature is an important factor in the development of black shank in the field.

The Effect of Certain Environmental Factors on the Germination of Florida Cigar-wrapper Tobacco Seeds.—R. R. KINCAID.

The cardinal temperatures for germination are approximately 10°, 24°, and 34° C. A daily alternation between 20° for 8 hours and 30° for 16 hours produces more rapid germination than a constant temperature of 24°. Oxygen concentrations of 60 per cent and higher are unfavorable for germination. The apparent isoelectric point of the seeds is about pH 6.8, as shown by bimodal curves of germination and water absorption and by ion-adsorption experiments. All of the 19 samples of seeds tested required light for germination at constant temperature. The amount of visible radiation required is of the order of 10–11 gram-calorie per seed. The seeds must be imbibed with water at the time they are exposed to light, but the effect of light is not reversed by subsequent drying. The effect of light on imbibed seeds is independent of the temperature of the seeds at the time of exposure. The requirement of light for germination may be offset by a sharp daily alternation of temperature during incubation.

Field Studies of Fusarium Wilt of Cotton.—C. D. SHERBAKOFF AND G. M. STONE.

A study of the development of fusarium wilt of cotton as affected by soil conditions was begun in western Tennessee in the summer of 1934. Data thus far obtained indicate that there is a correlation—most likely an indirect one—between the topography, the pH value, and the amount of organic matter in the soil on the one hand, and development of the wilt on the other. The greater number of wilted plants were found on the higher elevations, where the soil was less acid and its organic content low.

Effect of Crown Gall, Hairy Root, and Woolly Aphids on Apple Trees in the Orchard.—C. D. SHERBAKOFF AND J. A. MCCLINTOCK.

A test orchard of 1200 apple trees was set out at Jackson, Tennessee, in 1923, to determine what effect the presence of crown gall, hairy root, and woolly-aphid knot on nursery stock at planting time have upon the trees in the orchard. The nursery trees were grouped according to their condition and were set out without being fumigated or disinfected. Notes taken 1, 3, and 6 seasons later show apparently significant differences among the groups. Compared with trees, apparently healthy at planting time, there were 4 or more times as many dead, or dead and dying, among those with large crown galls or

extensive development of woolly-aphid knots, and 2 or more times as many among those with hairy roots, small crown galls, or slight development of woolly-aphid knots.

Control of Sweet-potato Stem Rot in West Tennessee.—G. M. STONE.

A survey was made in 1934 of the factors contributing to sweet-potato stem-rot infection, in typical fields of Weakley County—the leading sweet-potato county of Tennessee. During the same season and under the same field conditions, the crop on 2 farms was grown under the supervision of the writer, where certain necessary methods of stem-rot control were employed. The survey and test show, (1) that the best control of stem rot is obtained where new fields were set with Bordeaux-dipped slips produced in new beds, from certified, properly graded, and disinfected seed; and (2) that the factors contributing most to infection are, in the order named, old beds, contaminated fields, contaminated and ungraded seed.

Lespedeza sericea Stem Blight.—J. K. UNDERWOOD.

In 1933 and 1934 considerable loss from "stem blight" was noted on *Lespedeza sericea* in eastern Tennessee. The affected plants show a general yellowing, and the bark becomes shrunken and discolored. A Phoma-like organism has been found constantly associated with the disease and is suspected of being the cause of it. Positive infections were obtained when inoculations of disease-free seedlings were made with the fungus. The differences in the symptoms, however, indicate the possibility that the disease is more complex, or of a different causation, than one may conclude from the findings.

Black Root Rot of Apple.—J. O. ANDES.

Black root rot of apple has been found scattered throughout Tennessee. Only *Xylaria mali* Fromme has been isolated from or found in connection with the diseased roots. Preliminary tests and observations suggest that development of the disease can be arrested by sterilizing the immediate area of diseased trees. A treatment with a combination of steam and formaldehyde appears to be the most economical method tried for this purpose. Observations indicate a possibility of nursery trees introducing the disease.